JOINT EFFECTS OF MATERNAL METABOLIC CONDITIONS AND PLASMA BRANCHED-CHAIN AMINO ACIDS ON CHILD RISK OF AUTISM SPECTRUM DISORDERS (ASD): EVIDENCE OF SEX DIFFERENCE

by

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ABSTRACT

Background: Maternal obesity and diabetes are known risk factors for the development of child autism spectrum disorder (ASD). The association of other maternal metabolic disorders with ASD have been much less studied. Elevated branched-chain amino acids (BCAAs) are also associated with metabolic disorders and lower BCAAs have been linked to ASD. This dissertation sought to explore the role of maternal plasma BCAAs in the pathway from maternal metabolic disorders to child ASD and whether the associations differed by child's.

Methods: This study leveraged the Boston Birth Cohort (BBC), an ongoing prospective cohort study in the Boston area representing a predominantly urban, low-income, minority population. Of over 3,000 mother-infant pairs from the BBC prospectively from birth since 2004, this dissertation analyzed 864 with pertinent data. Maternal lipids were measured using standard clinical methods, and targeted BCAA metabolites were quantitatively profiled using liquid chromatography-tandem mass spectrometry (LC-MS/MS), using plasma samples collected 24-72 hours postpartum. A composite BCAA score was created using factor analysis and dichotomized at the median. Maternal obesity and diabetes were combined into one binary variable (ob/DM), and cholesterol subtypes were dichotomized using clinical cut-points. A multiple maternal metabolic disorders score was developed and dichotomized into (MMD<3 vs. ≥3). Logistic regression was used to explore the association between maternal BCAAs and child ASD risk and the potential role of BCAAs as mediators. Joint effects between BCAAs and maternal metabolic conditions (ob/DM (n=864), low high-density lipoprotein cholesterol (HDL-C) (n=829), and multiple metabolic disorders (MMD) (n=829)) as well as child's sex were also explored for both

ASD and other developmental disorders. Lastly, joint associations between all three risk factors – BCAAs, maternal metabolic conditions, and child's sex – were examined.

Results: While maternal BCAAs alone were not associated with child risk of ASD and did not mediate the association between maternal metabolic disorders and ASD, they were jointly associated with maternal ob/DM on child ASD risk (adjusted BCAA score OR 2.35, 95% CI 1.21, 4.55). While maternal HDL-C alone was not associated with child ASD risk, HDL-C and child's sex had a joint effect on this risk. Among mothers with low HDL-C, elevated BCAA score was associated with higher risk of child ASD (OR 4.67, 95% CI 1.33, 16.36; $p_{interaction}$=0.006). Maternal MMD and above median BCAA score synergistically increased the risk of ASD (adjusted OR: 3.20, 95% CI: 1.65-6.18; $p_{interaction}$= 0.019). Finally, the risk of ASD was the greatest among male children with mothers with elevated BCAAs and metabolic disorder(s) compared to other groups.

Conclusions: Maternal metabolic disorders had joint effects with elevated maternal plasma BCAAs and with male sex on child risk of ASD, and the risk was greatest among children with all three risk factors. These findings, raise the prospect of early risk assessment and primary prevention of ASD.

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ABBREVIATIONS

ASD: autism spectrum disorder
ADHD: attention deficit hyperactivity disorder
BBC: Boston Birth Cohort
BCAA: branched-chain amino acid
BCKDC: branched-chain α-keto acid dehydrogenase enzyme complex
BMC: Boston Medical Center
DM (or T2DM): type 2 diabetes mellitus
DSM: Diagnostic and Statistical Manual of Mental Disorders
GDM: gestational diabetes
HDL-C: high-density lipoprotein cholesterol
LBW: low birthweight
LC-MS/MS: liquid chromatography tandem mass spectrometry
LDL-C: low-density lipoprotein cholesterol
MIA: maternal immune activation
MMD: multiple metabolic disorders
mTOR: mammalian target of rapamycin
Non-HDL-C: non-high-density lipoprotein cholesterol
Ob/DM: obesity/diabetes
OR: odds ratio
RERI: relative excess risk due to interaction
ROS: reactive oxygen species
TC: total cholesterol
TCA cycle: tricarboxylic acid cycle
TD: typically developing
TG: triglycerides

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CHAPTER 1 INTRODUCTION

Autism spectrum disorders (ASDs) are characterized by deficits in social interaction and communication, and restricted or repetitive behaviors and are often accompanied by co-occurring conditions, including attention deficit hyperactivity disorder (ADHD), intellectual disability, and anxiety and mood disorders, among others.[1] The etiology of ASD is complex and though it is highly heritable, various environmental factors have also been implicated.[2] There is building evidence that the developing brain of the fetus can be influenced by an inflammatory maternal environment and immune activation.[3] Metabolic conditions, including obesity, diabetes, dyslipidemia and hypertensive disorder can be considered environmental stressors giving rise to oxidative stress and inflammation.[3,4]

The present research was motivated by recent findings from the Boston Birth Cohort (BBC), which have brought to new light on the importance of maternal metabolic disorders on child's risk of ASD. The BBC is an ongoing birth cohort following mother-infant pairs prospectively from birth up to age 21 years, representing a sample of urban, low-income, minority population in the United States. An earlier publication from this cohort has shown that maternal obesity and diabetes mellitus are independently as well as jointly associated with an increased risk of child development of ASD.[5] This has been replicated by studies in diverse populations.[6,7] Maternal hypertension has also been associated with child ASD.[4] There is less known about the associations between maternal cholesterol and child risk of ASD, though another recent publication from the BBC reported an association between maternal cholesterol, specifically lower levels of high-density lipoprotein cholesterol (HDL-C) and higher levels of triglycerides,

and attention deficit hyperactivity disorder (ADHD), a commonly co-occurring condition with ASD.[8]

To date, the molecular mechanisms underlying associations of maternal metabolic disorders and child ASD risk remain unclear. This study focuses on branched-chain amino acids (BCAAs), comprised of leucine, isoleucine, and valine for several reasons. First, BCAAs are specifically associated with obesity and diabetes, and at elevated levels they are predictive of incident type 2 diabetes.[9,10] Another line of research shows that an altered amino acid metabolite profile is a signature of ASD in laboratory and clinical studies, and is mainly found in lower levels in children with ASD than in TD children.[11,12] BCAAs are essential amino acids that have important direct and indirect signaling roles, including activation of the mechanistic target of rapamycin (mTOR).[13] The mTOR signaling pathway is also activated by insulin-like growth factor 1 (IGF-1) and promotes synaptic protein synthesis; thus its dysregulation has been implicated in neurocognitive abnormalities, including ASD.[14] However, major gaps exist, especially since most studies to date are cross-sectional by design, and there are no prospective birth cohort studies examining the inter-relationships of maternal metabolic disorders, maternal BCAAs, and child risk of ASD.

This study attempted to bring together several lines of research under a life course framework to better understand early life origins of ASD. It also sought to understand the underlying mechanisms of the male preponderance in ASD prevalence.[15] The goal of this research is to understand the pathways underlying the associations between maternal metabolic conditions and subsequent diagnosis of ASD in the child and the role BCAA play in this relationship. Specifically, the objectives were:

1. To explore the role of maternal plasma branched-chain amino acids (leucine, isoleucine,

and valine) in the pathway from maternal obesity/diabetes to child ASD.

Hypotheses:

- Maternal BCAAs either mediate or have joint effects with maternal ob/DM in the relationship between maternal ob/DM and child ASD risk.
- The risks from the associations above are higher in males than in females.

2. To assess the association between maternal cholesterol and child ASD and explore the role of maternal plasma branched-chain amino acids in the pathway from maternal dyslipidemia to child ASD.

   Hypotheses:

   - Low maternal high-density lipoprotein cholesterol (HDL-C), high maternal low-density lipoprotein cholesterol (LDL-C), and high maternal non-HDL-C, are associated with child ASD risk
   - Maternal BCAAs either mediate or have joint effects with maternal lipids in the relationship between maternal lipids and child ASD risk
   - The risks from the associations above are higher in males than in females.

3. To assess the association between maternal multiple metabolic disorders (MMD) and child ASD and explore the role of maternal plasma branched-chain amino acids in the pathway from maternal MMD to child ASD.

   Hypotheses:

   - Maternal BCAAs either mediate or have joint effects with maternal MMD in the

relationship between maternal MMD and child ASD risk

- The risks from the associations above are higher in males than in females.

This dissertation is organized into chapters. Chapter 2 presents the background and significance for the present topic, focusing on the burden, risk factors, maternal metabolic disorders, immune dysregulation and neuroinflammation, mitochondrial and oxidative stress, branched-chain amino acids, and sex differences. In Chapter 3, the data collection methods and study setting are described. This research study was nested within a larger prospective birth cohort study examining early life origins of pediatric and adult diseases. Chapters 4, 5, and 6 present the results of each of the specific aims outlined above. Chapter 4 explores the relationship of maternal BCAAs with ASD in conjunction with maternal obesity and diabetes and sex of the child. Chapter 5 investigates the association between maternal cholesterols and child ASD and evaluates joint effects of sex and the BCAAs with dyslipidemia. Chapter 6 then examines the relationship between MMD and ASD and again evaluates joint effects with BCAAs, stratifying by sex. Interactions between the maternal metabolic conditions and BCAAs as well as sex are also explored. Finally, in Chapter 7, the overall study findings are discussed along with strengths and limitations. The implications of the findings and future work are also discussed.

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CHAPTER 2 LITERATURE REVIEW

BACKGROUND

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired cognitive abilities that range from mildly to severely disabling and the diagnosis is virtually irreversible. The current prevalence estimate from the Center for Disease Control (CDC) in the United States is 1 in 59.[1] In 2010, there were 52 million cases of ASD worldwide, making it the leading cause of disability in children under five among all mental health disorders (measured by years lived with disability (YLD)).[2] The prevalence of ASD is reportedly similar across all regions of the world differing only slightly by socioeconomic status, race, and ethnicity. However, the most recent surveillance report from the Autism and Developmental Disabilities Monitoring (ADDM) network reported a higher prevalence among non-Hispanic white children than non-Hispanic black children and both of these groups had higher prevalence than Hispanic children.[1] This discrepancy may be reflective of poorer access to resources and diagnosis among minority and low-income populations.

In 2015, a study estimated that the total economic burden of ASD, including direct and indirect medical costs and productivity costs, in the United States was around $268 billion for that year, which exceeded the economic burden of hypertension and stroke.[3] Given the steady incline in the prevalence, they forecasted the economic burden at around $461 billion for 2025, far surpassing that of ADHD and diabetes in the country. Though children with ASD are eligible for Medicaid coverage in the United States, access to care varies by neighborhood, race/ethnicity, and affluence.[4]

The etiology of ASD is quite complex and thought to include genetic, environmental, and epigenetic factors.[5] There is also no cure or highly effective primary preventive measure for ASD. Decades of research have gone into understanding both facets of this enigmatic disorder. Early interventions have shown the most promise to increase IQ, language skills, and social behavior in diagnosed children.[6] However, as primary prevention is always preferred over secondary prevention and therapeutic efforts, there is a need for investigation of early risk assessment, prediction, and primary prevention before or during pregnancy.

DEFINITION AND DIAGNOSIS

The current diagnostic criteria used in the United States are found in the fifth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), released by the American Psychiatric Association in May 2013.[7] Diagnosis is made using a developmental screening and a comprehensive diagnostic evaluation by a trained clinician; there is no biological marker that has been determined as an indicator for the disorder. The current criteria include two domains where sustained deficits must be shown in people with ASD: "persistent social communication and social interaction" and "restricted and repetitive patterns of behavior." Clinicians will also diagnose the level of severity based on "level of support" required for the individual to carry on with their daily lives. These tests are considered reliable for diagnoses at two years of age but ASD can be detected as early as 18 months and sometimes younger. Under the previous DSM (DSM-IV), conditions such as Asperger syndrome were categorized separately from autism; these are now included under a broad spectrum. ASD affects individuals in different ways. People with ASDs may have atypical ways of communicating and interacting than most other people and may have learning disabilities. They may also display advanced abilities in a specific area such as drawing or mathematical ability.

CURRENT UNDERSTANDING OF ASD ETIOLOGY

Brain Structure and Function: Rather than having atypical brain regions, ASD individuals are thought to have differences in the neural connectivity, both short- and long-range, compared to typically developing individuals.[8] This is in part due to molecular genetics of synaptic proteins, cell adhesion molecules, and the imbalance of excitatory and inhibitory neurotransmitters. The PI3K/AKT/mTOR signaling pathway plays a central role in synaptic protein synthesis,[9] and its dysregulation results in many behavioral abnormalities, which may contribute to the etiology of ASD.[10] PTEN negatively regulates the PI3K/AKT/mTOR pathway, and thus, deletion of the PTEN gene results in an overactive PI3K/AKT/mTOR pathway and autism-like behaviors in mice.[11]

Genetics: Recent estimates suggest that genetic factors explain around 40-50% of ASD risk.[12] Some studies report even higher heritability estimates at around 95%.[13] However, there are several hundred genes implicated as risk factors of ASD as opposed to one or a few.[14] In one study, monozygotic twins had a higher concordance rate (80%, 95% CI 51.9-95.7%) than dizygotic twins (13.6%, 95% CI 2.9-34.9%).[15] Male sex is also a strong predictor, associated with ASD in about a three to one ratio between males and females.[16]

Rare genetic mutations, including *de novo* and inherited account for an estimated 10-30% of ASDs. This includes the approximate 10% of cases, ASD is the result of a genetic syndrome.[17] For example, up to 60% of males with Fragile X Syndrome cases, caused by a known mutation in the *FMR1* gene, show signs of ASD.[17,18] There are several other genes that have cumulatively been implicated in ASD etiology, though no common gene loci have been identified for ASD, as is the case for schizophrenia.[19]

Epigenetics: Epigenetics, including DNA methylation and histone methylation and acetylation, have been shown to play a large role in the etiology of autism.[20] Autism is associated with several disorders that are caused by mutations in genes that regulate the expression of other genes.[21] Furthermore, several parental imprinting regions on specific chromosomes have also been reported to be associated with autism.[22,23] A recent report examined the joint analysis of genotype with DNA methylation data from cord and peripheral blood in relation to ASD phenotypes and found that together, they provide more insight than each factor alone.[24] Furthermore, prenatal stress from a variety of sources discussed below can also induce epigenetic changes owing to ASD pathology.[25]

Sex differences: Autism is a sexually dimorphic disorder with an approximate four-fold higher prevalence in males than females.[45] There is evidence of the association between gonadal hormones in infancy and sex-specific differentiation of brain morphology and function as well as neurochemical differences. This difference in sex hormones is also been shown to be associated with development of language and motor skills,[46,47] behaviors such as aggression, as well as the risk of development of autism.[48] The hypothalamic-pituitary-gonadal (HPG) axis of the fetus of both sexes is active during mid-gestation, repressed later in pregnancy by placental hormones, and reactivated postnatally, peaking at around three months and declining through six months of age. However, in girls, follicle stimulating hormone (FSH) levels stay elevated until 3-4 years of age.[49] The main estrogen in females, estradiol, was not only found to have receptors on the mitochondria, but also was found to signal antioxidant pathways in the organelle.[50] Additionally, pre-pubertal girls have approximately eight times more estrogen than pre-pubertal boys.[51] Estrogen is protective against glutamate-driven neurotoxicity, which is consistently shown in the literature as reviewed by Pastural et al (2009).[45,52] Thus, this might be one mechanism protecting

females in infancy from developing oxidative stress-related disorders including ASD and ADHD. This is also evidenced by sex differences in aging as females have greater longevity than males; females have lower levels of oxidants present in their mitochondria than males due to upregulation of antioxidant enzymes via estrogen signaling of nuclear genes directed toward the energy producing organelle. The evidence is more equivocal for FSH. A study in rats found that FSH was protective against oxidative stress and apoptosis in pre-ovulatory follicles.[53] However, a later study showed that though apoptosis of mouse ovarian granular cells was inhibited with FSH treatment, oxidative stress levels in the cells remained the same.[54]

Immune Dysregulation and Neuroinflammation: A proposed underlying mechanism of ASD is the dysregulation of glutamatergic neurotransmission in the brain, which causes increased excitation of pro-inflammatory cytokines leading to neuroinflammation.[55] Over the past few decades, there has been growing evidence linking maternal inflammation and autism, especially through the diet, due to both over- and under-nutrition. Inflammation caused by infection and environmental exposures has also been studied linking maternal stress during gestation to a host of neurodevelopmental disorders including autism, schizophrenia, and attention deficit hyperactivity disorder.[56] Maternal inflammation or "maternal immune activation" (MIA) has been shown to be associated with neurodevelopmental disorders.[57] The theory is that pro-inflammatory markers in the maternal environment due to infection or other stressors can enter fetal circulation through the placenta and cross the blood-brain-barrier, causing excessive neuronal growth and abnormal neural plasticity.[58] Chronic low-grade inflammation can be caused by long-term stresses to the body, including environmental stressors such as sun exposure and air pollution, dietary exposures including high-fat diets, and chronic health conditions like diabetes or obesity.

Mitochondria and Oxidative Stress: Beta-oxidation occurring in the mitochondria during the breakdown of fatty acids produces reactive oxygen species (ROS) and reactive nitrogen species (RNS) in small quantities.[59] In normally functioning mitochondria, these reactive species are regulated and contained for the most part. However, an imbalance in input can cause disruption to this regulation, causing leakage of the free radicals, which can cause oxidative stress by reacting to and thus damaging cellular components including DNA. This in turn leads to an inflammatory response, which can remain chronic and unresolved at low levels if the source of this oxidative stress is not removed. An example is in the evidence that obesity (associated with an excess of lipids) is a risk factor for other chronic and inflammatory diseases like type 2 diabetes and cardiovascular disease (CVD).[60]

In order to compensate for the increase in ROS and RNS, mitochondria become overactive.[61] Thus, high levels of reactive species resulting in oxidative stress can further exacerbate the beta oxidation process leading to mitochondrial dysfunction (MD). This affects ATP synthesis, which in turn decreases glutathione (GSH) production. A meta-analysis by Rossignol and Frye (2012) found that 30% of children with ASD had biomarkers for MD[62] and a follow up study by Frye found up to 50% of children with ASD had biomarkers of MD.[63]

Though a low level of reactive species is required for normal cellular functioning, higher levels of exposure could lead to "oxidative stress," causing functional impairments via damage to cellular membranes, proteins, and DNA.[59] The ASD brain is thought to be particularly vulnerable to damages caused by oxidative stress. A study showed that plasma levels of glutathione (GSH), and the ratio of reduced to oxidized glutathione, an indicator of antioxidant capacity and redox homeostasis, were significantly decreased in children with ASD compared to controls (52). Of all antioxidants produced by the body, GSH is present at the highest concentration, and is thus

necessary in the defense against damage from oxidative stress. In a GSH-deficient state, a cell's capacity to buffer excessive reactive species is decreased due to faster depletion of GSH leaving the cell vulnerable to the damage. One theory for an etiologic mechanism of ASD is exposure to environmental toxins that deplete the GSH pool.[59] As GSH is a product of the methionine metabolism, disruptions in the methionine/ homocysteine/ folate pathway, including folate deficiency, can also affect GSH production.

Environmental factors: Mother's diet, nutritional status, and infection during pregnancy are all known risk factors. For example, maternal obesity and diabetes mellitus (DM) have been shown to be independently associated with a two-fold increased risk of autism.[26] Obesity and DM combined had an even higher risk (hazard ratio 3.91, 95% CI 1.76–8.68) as did obesity and gestational diabetes (GDM) (hazard ratio 3.04, 95% CI 1.21–7.63). Nutritional deficiencies have also been implicated in the risk of ASD, which may also occur in overweight and obese individuals. Vitamin D and folate are important nutrients for proper brain development during gestation. The direct link between vitamin D deficiency and ASD is not yet confirmed but has grounding in several observational studies and open label trials as evidenced in a recent review.[27] The review reported higher maternal concentrations of vitamin D were associated with lower risk of autism-related traits in children. Along with decreasing the risk of neural tube defects, folate supplementation during pregnancy has been shown to be protective against the risk of ASD.[28] However, a recent study has shown that extremely high folate and vitamin B12 levels during pregnancy can also be a risk factor for ASD, resulting in a "U" shaped relationship.[29] Insufficient fatty acids, including omega-3 DHA and EPA, can also lead to abnormal development of the brain and have also been associated with ASD.[28]

Gestational zinc deficiency is another known risk factor for ASD.[30] Lipopolysaccharides (LPS), an endotoxin in bacteria, plays a role in autism as it leads to hypozincaemia, causing gestational zinc deficiency. Eating a high-fat diet has been linked to increased levels of LPS in healthy humans and mice.[31] A study done in rats to examine the effects of zinc supplementation with high LPS found that impairment induced by LPS was reduced to the level of control pups when the mothers were also given zinc.[30] This further led to reduced level of BDNF to the level of controls as well as prenatal LPS increases levels of BDNF, even as adults. ASD has also been shown to be associated with other micronutrient deficiencies apart from zinc.[32] Epigenetic changes may occur when these micronutrients are lacking or altered during pregnancy, including folic acid and vitamin B12. Deficiencies in these nutrients can lead to preterm birth as well as behavioral disorders including autism. These findings may support methods of preventing micronutrient deficiency as well as sanitation and hygiene to prevent bacterial infection.

Older maternal and paternal age are both known risk factors for ASD.[33-35] Low birthweight and being born prematurely are also known biological risk factors.[36-38] One theory behind the association between preterm birth and ASD is via an event or chronic condition triggering an inflammatory fetal environment that causes the prior to term birth and also leads to microglial activation in the fetal brain, leading in turn to abnormal synapse formation and brain development.[38]

Other environmental factors, including air pollution,[39,40] environmental toxins,[5,41] psychological stress,[25] and migration[37,42] have all been identified as maternal risk factors, which likely act via epigenetic mechanisms.[28] At the cellular level, there are multiple mechanisms at play that may be useful in studying the etiology of the disorder. Cellular dysfunction, including oxidative stress, mitochondrial abnormalities, neuroinflammation, and abnormal synaptic plasticity, are generally

seen in ASD patients and can play a role in providing several biomarkers for the study of the disorder.[43] For example, neurotoxicants, including air pollution, induce neuroinflammation and oxidative stress, and disproportionally affect males over females due to several protective mechanisms in females, including the neuroprotective effects of estrogen and progesterone.[44] There are also several preclinical studies reporting the effects of neurotoxicants as well as psychological stress of dams on autism-like characteristics in the offspring.[25,44] Critically, a better understanding of these factors, whether or not they are in a causative pathway, should lead to a better understanding of means and avenues with which to intervene to improve behavioral and cognitive outcomes.

Obesity and Diabetes: There is building evidence that the developing brain of the fetus can be influenced by an inflammatory maternal environment due to diet or obesity. For example, rats exposed to a high-fat diet before and during pregnancy had increased dam weight as well as leptin, CRP, and IL-6 concentrations, which in turn activated the maternal immune system and led to increased expression of cytokines in the offspring brain at birth. Long-term changes in brain function and behavior of the offspring due to high dam weight and a high-fat diet were "strikingly similar to that observed following an early-life bacterial infection."[64]

Maternal obesity and diabetes mellitus (DM) have been shown to be independently as well as jointly associated with an increased risk of child development of ASD.[26] The most recent meta-analysis on pre-pregnancy and pregnancy body mass index (BMI) reported increased risk of offspring ASD among overweight mothers (OR (odds ratio): 1.16; 95% CI 1.05-1.27) and obese mothers (OR: 1.41; 95% CI 1.19-1.67) compared to normal weight mothers.[65] A recent meta-analysis on maternal diabetes reported a 48% increased risk of child ASD in mothers with diabetes compared to those without the condition (RR (risk ratio): 1.48; 95% CI: 1.26–1.75.[66]

Furthermore, the subset of moderate and high quality studies resulted in a 62% increased risk (RR: 1.62; 95% CI: 1.35–1.94).

The presently held hypothesis in scientific literature of the underlying mechanism behind the association between these metabolic conditions and child ASD risk is maternal chronic, low-grade inflammation leading to oxidative stress.[43,59] The neonatal brain is susceptible to cytokines and other pro-inflammatory markers that can cross the placenta as well as the blood brain barrier, affecting the developing brain of the fetus. Another hypothesis is excess brain growth during the critical period of catch-up growth for children born with low birthweight (LBW), with maternal obesity being a risk factor for LBW babies.[67] An animal study also showed maternal inflammation contributed to brain overgrowth and autistic features in offspring.[68]

Like obesity, maternal diabetes also creates an inflammatory environment in utero, which has been associated with ASD in offspring.[66] Maternal hyperglycemia, as a result of diabetes, is linked to oxidative stress and can also lead to hyperinsulinemia in the fetus, causing increased oxygen consumption and hypoxia. Both oxidative stress and hypoxia are known risk factors for ASD. Further, if the mother is both obese and diabetic, there can be a potential interaction, presenting an even higher risk than each alone

Cholesterol and Vitamin D: At normal levels, cholesterols are important components of cellular membranes, regulating permeability and synapse formation in the brain. Approximately 25% of the cholesterol in the body is found in the brain and the myelin sheath, which protects neuronal axons, contains 70% of brain cholesterol.[69] They also play important roles in the development of the fetal brain as they are involved in cellular membrane and steroid hormone formation. Certain cholesterols may cross the placenta affecting the concentrations in the womb, and aberrant concentrations have been shown to be associated with adverse outcomes in the

offspring. Fragile X syndrome, closely related to ASD, is marked by low levels of myelin.[70] Low maternal high-density lipoprotein cholesterol (HDL-C) and high triglycerides were also found to be associated with ADHD in the BBC.[71] Finally, both low and high total cholesterol were associated with preterm birth.[72] Both ADHD and preterm birth are highly associated with ASD.

Cholesterols are precursors of all steroid hormones except for vitamin D. However, vitamin D and cholesterol are both derived from the same molecule, 7-dehydrocholesterol (7DHC).[70] Steroid hormones have been associated with ASD and other mood disorders and there is building evidence implicating vitamin D in ASD etiology.[27,73,74] The genes expressing serotonin, oxytocin, and vasopressin each contain a vitamin D response element (VDRE) and require vitamin D to activate them.[74] All three hormones are reported to be dysregulated in ASD and are also regulated by cholesterol. A recent study showed that low concentrations of 25-hydroxyvitamin D in the first trimester of pregnancy had significantly increased levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C) and total cholesterol to HDL-C ratio compared to pregnant women with adequate levels of the vitamin.[75]

Metabolic Syndrome: Metabolic syndrome is a loosely defined term for a cluster of co-occurring metabolic risk factors of type 2 diabetes and cardiovascular disease.[76] Though there is no obligatory component, metabolic syndrome typically comprises elevated blood pressure, dyslipidemia, hyperglycemia, and central obesity. We and others have shown maternal obesity and diabetes are associated with child risk of ASD.[26,65,66] The prevalence of pre-pregnancy hypertension was also higher among mothers who had children with ASD, and together with obesity and diabetes, was associated with a higher likelihood of having a child with ASD.[77]

Amino Acids: Extensive evidence points to differences in levels, metabolism, and functionality of amino acids in the autistic brain. Most of these studies consider glutamate, the major

excitatory neurotransmitter and γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter as reviewed by Zheng et al (2017).[78] This review also highlights the function and significance of other neuroactive amino acids, including tryptophan, serine, glycine, and the branched-chain amino acids (BCAAs) – leucine, isoleucine, and valine. Ten studies report higher glutamate concentrations in blood plasma of individuals with ASD compared to healthy controls, while one reported a lower concentration. However, while three neuroimaging studies also found higher glutamate in the brain of individuals with ASD compared to control, one study reported lower glutamate and another found no difference. All three studies of urine metabolites found lower glutamate concentrations in individuals with ASD. Two studies in plasma and one in urine found higher GABA levels but all five neuroimaging studies reported lower levels of GABA in the brain in individuals with ASD. Similarly, all other amino acids also had inconsistent findings, except leucine and isoleucine, which were lower in individuals with ASD compared to controls in all reports of plasma, urine, and cerebrospinal fluid.

Branched-chain amino acids: The BCAAs – leucine, isoleucine, and valine – are essential amino acids and the most abundant in animal proteins.[79] They are involved in protein synthesis, metabolic and glucose homeostasis, and are key nitrogen donors in the form of glutamate, glutamine, and alanine.[80] Glutamate is the major excitatory neurotransmitter and also the precursor for GABA, the major inhibitory neurotransmitter. Leucine is also important in cellular signaling and is a potent activator of the mammalian target of rapamycin (mTOR). mTOR is a kinase that regulates a vast network of cellular mechanisms, including insulin release, lipid synthesis, and promotion of protein synthesis in the periphery as well as in the CNS. BCAAs have been studied as biomarkers associated with obesity,[81,82] diabetes,[80,81,83] inflammation and the immune system,[80,84] and cholesterol metabolism.[85,86]

BCAAs are first metabolized via transamination by branched-chain aminotransferase (BCAT) into their respective branched-chain α-keto acids (BCKAs).[80] The BCKAs then undergo oxidative decarboxylation by the branched-chain α-keto acid dehydrogenase enzyme complex (BCKDC) to produce their respective branched-chain acyl-CoA derivatives. Maple syrup urine disease (MSUD) is the most well-known BCAA-related disorder caused by mutations in the BCKDC protein, and can cause severe neurological damage.[84] The branched-chain acyl-CoA derivatives are then further catabolized until they finally enter the tricarboxylic acid (TCA) cycle or serve as precursors for lipogenesis or gluconeogenesis.[80] Imbalance of TCA cycle inputs can disrupt beta oxidation in the mitochondria and lead to mitochondrial dysfunction, implicated in ASD etiology.[43,59] As mentioned in the section above, BCAA concentrations are consistently reported to be lower in ASD individuals compared to typically developing individuals.[78,87,88] There are no intergenerational studies, to our knowledge, investigating maternal BCAA concentrations with child risk of ASD.

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CHAPTER 3 METHODOLOGY

DATA SOURCE

The Boston Birth Cohort (BBC) was established in 1998 at the Boston Medical Center by principal investigator, Dr. Xiaobin Wang and the study is ongoing. The BBC was initially designed as a molecular epidemiologic study to investigate risk factors for preterm birth and low birthweight in a low income, minority population in the Boston area. Employing a rolling ongoing enrollment, the study has successfully recruited over 8,600 mother-infant pairs to date with over a 90% participation rate among eligible mothers approached by the study staff. Mothers were recruited 24-72 hours post-partum, and written informed consent was obtained from the participants. Exclusion criteria included multiple births, pregnancies due to *in vitro* fertilization, babies with chromosomal abnormalities or major birth defects, and children with other developmental disorders.

Beginning in 2003, a subset of children receiving care at the Boston Medical Center were enrolled in the Children's Health Study and followed up during well-child visits.[1-3] In 2003, the Boston Medical Center implemented the Electronic Medical Records (EMR) system, from which the BBC was able to collect information on physician diagnoses on children based on the International Classification of Diseases, Ninth Revision or Tenth Revision (ICD-9 or ICD-10). This information became a rich source of clinical data for the BBC and included EMR of inpatient and outpatient units as well as the emergency and operating rooms and laboratory tests. The BBC has obtained EMR data from each postnatal visit during 2003 – 2016. The Children's Health Study has over 3,000 participants, from which our study samples were selected. Only mothers with metabolite measurements and children receiving continued care at the BMC were

included in the present study. Both the initial and follow-up studies were approved by the Institutional Review Boards (IRB) of the Johns Hopkins Bloomberg School of Public Health and the Boston University Medical Center. The exclusion criteria for the present study (Aim 1) is shown in Figure 3-1. Sample sizes for each aim are presented in Table 3-1 and maternal and child characteristics for included and excluded participants (from Aim 1) are compared in Table 3-2.

The power calculation reflects maternal branched-chain amino acid (BCAA) score for Aims 2 and 3 since these aims had the smallest sample size (n=829). The rate of ASD in the BBC is 10.4%, higher than the CDC reported rate,[4] this is in part due to the fact that the BBC is enriched by children with preterm and low birthweight, which are known risk factors of ASD.[5] With our sample size, H.

Figure 3-1. Flowchart of study sample included and excluded in the analyses

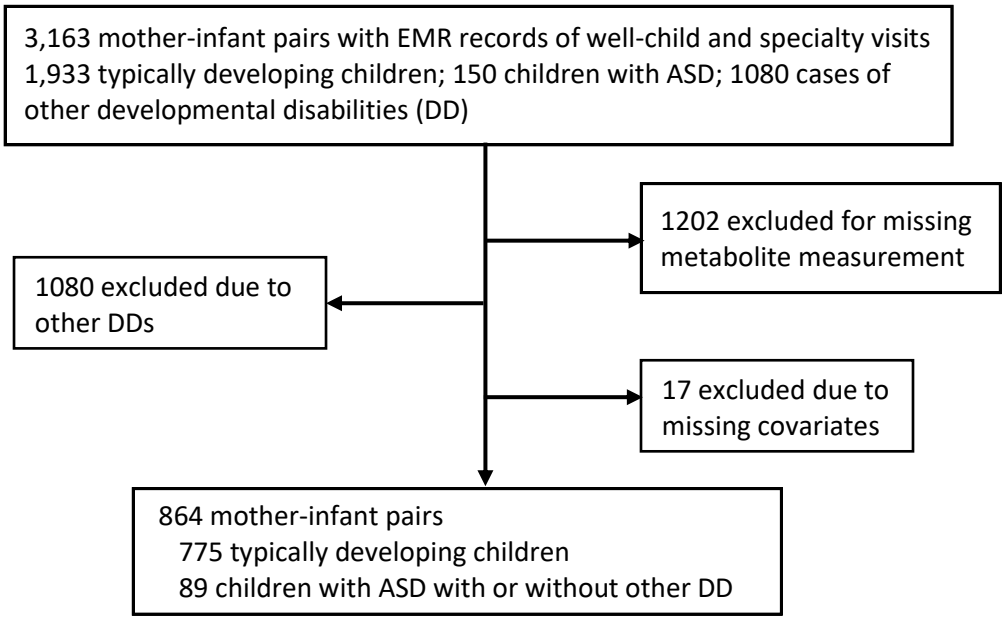

Table 3-1. Sample size

Sample Size	N	ASD	Typically Developing
Aim 1			
Maternal obesity/diabetes and BCAAs	864	89	775
Aim 2			
Maternal cholesterol and BCAAs	829	86	743
Aim 3			
Maternal multiple metabolic disorders and BCAAs	829	86	743

Table 3-2. Maternal and child characteristics between participants excluded and included in the analysis

Characteristics	Total, N (%)	Excluded, N (%)	Included, N (%)	P-value[a]
Total	3138 (100.00)	2274 (72.47)	864 (27.53)	
Maternal age (years), mean (SD)[b]	28.64 (6.50)	28.64 (6.48)	28.21 (6.51)	0.094
Nulliparous, n (%)	1337 (42.61)	958 (42.13)	379 (43.87)	0.379
Race or ethnicity, n (%)[c]				<0.0001
Black	1998 (63.67)	1387 (60.99)	611 (70.72)	
White	227 (7.23)	192 (8.44)	35 (4.05)	
Hispanic	701 (22.34)	540 (23.75)	161 (18.63)	
Other	212 (6.76)	155 (6.82)	57 (6.60)	
Maternal education, n (%)				0.973
Below college degree	2690 (85.72)	1949 (85.71)	741 (85.76)	
College degree or above	426 (13.58)	309 (13.59)	117 (13.54)	
Missing	22 (0.70)	16 (0.70)	6 (0.69)	
Maternal BMI, n (%)				
Mean (SD)	26.58 (6.65)	26.59 (6.67)	26.56 (6.60)	0.918
Normal weight (<25)	1452 (46.27)	1046 (46.00)	406 (46.99)	0.842
Overweight (25 - <30)	813 (25.90)	595 (26.17)	218 (25.23)	
Obese (≥30)	703 (22.40)	501 (22.03)	202 (23.38)	
Missing	170 (5.42)	132 (5.80)	38 (4.40)	
Maternal Diabetes[d]				0.099
No diabetes	2751 (87.67)	1980 (89.68)	771 (89.24)	
Diabetes	387 (12.33)	294 (12.93)	93 (10.76)	
Maternal smoking, n (%)[e]				
Never	2542 (81.01)	1812 (79.68)	730 (84.49)	0.001
Quit	241 (7.68)	186 (8.18)	55 (6.37)	
Continuous	336 (10.71)	267 (11.74)	69 (7.99)	
Missing	19 (0.61)	9 (0.40)	10 (1.16)	
Child's, n (%)				0.001
Male	1583 (50.45)	1187 (52.20)	396 (45.83)	
Female	1555 (49.55)	1087 (47.80)	468 (54.17)	
Gestational age, n (%)				0.001
Term (≥37 weeks)	2448 (78.01)	1712 (75.29)	736 (85.19)	
Late preterm (34-36 weeks)	306 (9.75)	238 (10.47)	68 (7.87)	
Early preterm (<34 weeks)	384 (12.24)	324 (14.25)	60 (6.94)	
Birthweight				<0.0001
≥2,500 grams	2275 (72.50)	1573 (69.17)	702 (81.25)	
<2,500 grams	863 (27.50)	701 (30.83)	162 (18.75)	

SD, standard deviation

[a]P-values were obtained from chi-square or t-tests; missing values for categorical variables incorporated with largest sized group

[b]Maternal age at time of delivery

[c]Black includes self-reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[d]Type II diabetes mellitus and/or gestational diabetes mellitus

[e]Never smokers were defined as mothers with no history of smoking six months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of six months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting six months prior to and throughout pregnancy

DATA COLLECTION

Mothers were recruited into the BBC within 72 hours post-partum and informed consent was obtained from the participants. A standardized questionnaire interview was conducted to obtain maternal demographic information and smoking status. A medical abstraction form was used to collect clinical data from maternal and newborn medical records, including parity, pre-pregnancy weight and height, pre-pregnancy diabetes, hypertensive disorders, pregnancy-related complications, gestational age, and birthweight. A non-fasted venous blood samples was also collected by antecubital venipuncture at this time. Blood plasma was analyzed for a lipid panel, including total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG). Targeted metabolites, selected for their associations with obesity and metabolic disorders, from the plasma were quantified using an LC-MS/MS at the Massachusetts Institute of Technology Broad Institute Metabolite Profiling Laboratory. ASD diagnosis was obtained from EMR records at every postnatal visit starting in 2003. This study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and Boston University Medical Center.

MEASURES

Primary outcome

The primary outcome of this investigation is the child's clinical diagnosis of ASD. ASD cases were defined by clinician diagnosed ICD codes in the EMR. Prior to October 1st, 2015, ICD-9 codes used for autism were 299.0, 299.00, 299.01, 299.8, 299.80, 299.81, 299.9, 299.90, or 299.91 and beginning on October 1st, 2015, ICD-10 codes were used (F84.0, F84.8, or F84.9).

The Diagnostic and Statistical Manual of Mental Disorders (DSM) is set by the American Psychiatric Association, with DSM-IV being released in 1994 and DSM-5 in 2013.[6] The DSM-IV included sub-diagnoses, including Autistic Disorder, Asperger Syndrome, Pervasive Developmental Disorder Not Otherwise Specified, and there were three areas of diagnostic criteria: social reciprocity, communicative intent, restricted and repetitive behaviors. In the DSM-5, there is only one diagnosis of Autism Spectrum Disorders and the diagnostic criteria have been condensed to two areas: social communication/interaction and restricted and repetitive behaviors. Symptom severity for each of these areas is also defined. Across both diagnostic criteria, the symptoms are required to be functionally impairing and must be present in early childhood, though they may not fully manifest until later under increasing social demands. The diagnoses of all ASD cases were obtained from electronic medical records after having been evaluated by highly trained staff the ASD evaluation center at the Boston Medical Center, who are in direct contact with primary care physicians center. Table 3-3 presents the ICD9 codes for our case definition of ASD.

Primary exposures/risk factors

The primary exposures are listed in Table 3-4. Maternal plasma total cholesterol, HDL, LDL, TG, and metabolites were measured using nonfasting blood samples obtained between 24-72 hours post-delivery. Table 3-5 outlines the components and their values that comprise the MMD score.

Table 3-3. List of ICD-9 and ICD-10 codes for the diagnosis of each developmental disorder

Developmental disorder	ICD-9 codes	ICD-10 codes
ASD	299.0, 299.00, 299.01, 299.8, 299.80, 299.81, 299.9, 299.90, 299.91	F84.0, F84.8, F84.9
ADHD	314.0, 314.00, 314.01, 314.1, 314.2, 314.8, 314.9	F90, F90.0, F90.1, F90.2, F90.8, F90.9
Developmental delays	315.0, 315.9	F81.0, R48.0, F81.81, F81.2, F81.89, F80.1, F80.2, H93.25, F80.4, F80.81, F80.0, F80.82, F80.89, F82, F88, F81.9, F89
Intellectual disabilities	317 – 319	F70, F71, F72, F73, F78, F79

Table 3-4. List of primary exposures

Primary exposures	Source	Definition
Aim 1 (N=864)		
Maternal obesity	Standardized interview	Pre-pregnancy BMI ≥ 30 kg/m^2
Maternal diabetes		
Type 2 diabetes	EMR	Pre-pregnancy ICD-9 codes 250.00–250.93
Gestational diabetes	EMR	ICD-9 codes 648.00 and 648.03
Maternal Plasma BCAAs & score	Collected within 1-3 days of birth; quantified via LC-MS/MS	Cut off at the median
Aim 2 (N=829)		
Maternal TC	Collected within 1-3 days of birth	Cut off at ≥240 mg/dL
Maternal HDL	Collected within 1-3 days of birth	Cut off at <50 mg/dL
Maternal LDL	Collected within 1-3 days of birth; calculated from Friedewald equation	Cut off at ≥160 mg/dL
Maternal non-HDL	Subtracted HDL-C from TC	Cut off at ≥190 mg/dL
Maternal Plasma BCAAs & score	Collected within 1-3 days of birth; quantified via LC-MS/MS	Cut off at the median
Aim 3 (N=829)		
Maternal obesity	Standardized interview	Pre-pregnancy BMI ≥30 kg/m^2
Maternal diabetes		
Type 2 diabetes	EMR	Pre-pregnancy ICD-9 codes 250.00–250.93
Gestational diabetes	EMR	ICD-9 codes 648.00 and 648.03
Maternal HDL	Collected within 1-3 days of birth	Cut off at <50 mg/dL
Maternal hypertensive disorders	EMR	Any one of the following: eclampsia, pre-eclampsia, chronic hypertension, gestational hypertension, or hemolysis elevated liver enzymes low platelet count (HELLP) syndrome)
Maternal Multiple Metabolic Disorders	A score comprising the above components	MMD cut-off ≥3; see Table 3-5 for full definition
Maternal Plasma BCAAs & score	Collected within 1-3 days of birth; quantified via LC-MS/MS	Cut off at the median

Table 3-5. Maternal Multiple Metabolic Disorders (MMD) Definition

Component	Points
Maternal BMI[a]	
Normal (<25 kg/m^2)	0
Overweight (25 - <30 kg/m^2)	1
Obese (≥30 kg/m^2)	2
Maternal diabetes[a]	
None	0
Gestational diabetes	1
Type 2 diabetes	2
Maternal HDL	
≥50 mg/dl	0
<50 mg/dl	1
Maternal hypertensive disorders	
None	0
Any[b]	1

Note: MMD is defined as a score of ≥ 3 with a total possible score of 6
[a]Mutually exclusive categories
[b]See Table 3-4 for definition of hypertensive disorders

Other covariates

Table 3-6 provides a list of pertinent pre- and peri-natal and child factors that could potentially confound the relationship between maternal obesity/diabetes, cholesterol, multiple metabolic disorders, BCAAs and ASD risk. These factors were adjusted for in the multivariate models.

Table 3-6. Maternal pre-pregnancy and child factors used as covariates in the models

Variables	Definition	Type
Maternal factors		
Maternal age	Maternal age at the time of enrollment	Continuous
Parity	Number of previous deliveries not including index pregnancy - nulliparous vs. multiparous	Binary
Maternal education	Below college degree vs. college or more	Binary
Maternal race/ethnicity	Black, White, Hispanic and other	Categorical
Smoking during pregnancy	Whether mother ever smoked 3 months before pregnancy/during pregnancy - never, quit, continuous	Categorical
Child factors		
Sex	Child's sex	Binary
Gestational age	Preterm (<37 weeks) vs. term delivery	Binary
Birthweight	Low birthweight (<2500 g) vs. normal birthweight	Binary

DATA ANALYSIS

Descriptive data analysis and derived variables: Exploratory data analysis was conducted to examine ranges and distributions for all key variables. The BCAA metabolites were inverse normally transformed to produce Gaussian distributions. The primary outcome, ASD diagnosis, was binary. The exposure variables obesity/diabetes, and cholesterols were treated as binary and BCAAs were examined as binary, categorical, and continuous. The BCAA score was created using factor analysis of the three BCAAs, leucine, isoleucine, and valine using the Anderson-Rubin Method.[7] Each BCAA and the BCAA score were dichotomized into "below median" and "above median." Chi-squared tests were conducted for binary and categorical variables and t-tests were conducted for continuous variables across the ASD and TD groups. Missing values for categorical covariate variables were incorporated into the largest sized group. Any un-detectable metabolite values were imputed with one-half the limit of detection. Since gestational age and birthweight were highly correlated, they combined into one categorical variable with four groups (1. full term (≥37 completed weeks of gestation) and non-LBW (≥2500g); 2. full term and LBW; 3. preterm and non-LBW; 4. preterm and LBW). All analyses were completed in Stata v14.0 (Stata Corporation, College Station, TX, USA).

Analysis for Aim 1

Due to the high correlation between the exposure variables, obesity and diabetes, the two were combined into one variable. This variable was analyzed as a binary variable (no obesity or DM vs either/both obesity or DM), as well as a categorical variable (1. no obesity nor DM; 2. either obesity or DM; 3. both obesity and DM). The associations between BCAAs and ASD were evaluated using multinomial logistic regression. The logistic regression model is represented by:

$$\ln(\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta 0 + \beta 1 \text{BCAA} + \varepsilon$$

We further tested for mediation and joint effects, including interaction. The hierarchical regression method was conducted for mediation analysis of BCAAs for the association between ob/DM and ASD.[8] Interaction was tested between BCAAs and ob/DM as well as BCAAs and child's sex employing tests for additive and multiplicative interaction and the relative excess risk due to interaction (RERI).[9] To examine joint effects of ob/DM with BCAAs, a categorical variable with four groups was created as follows:

Ob/DM	BCAA	New variable
0	0	0
0	1	1
1	0	2
1	1	3

The logistic regression model is represented by:

$$\ln (\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta_0 + \beta_1 \text{ob/DM}_0\text{BCAA}_{0i} + \beta_2 \text{ob/DM}_0\text{BCAA}_{1i} + \beta_3 \text{ob/DM}_1\text{BCAA}_{0i} + \beta_4 \text{ob/DM}_1\text{BCAA}_{1i} + \beta_c C_i + \varepsilon_i$$

where Y_i is the outcome for subject i, C_i are the set of covariates for subject i, and β represents the corresponding regression coefficient for each term. Subscripts 0 are for the condition being absent and subscripts 1 are for the condition being present.

With interaction term:

$$\ln (\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta_0 + \beta_1 \text{ob/DM}_0\text{BCAA}_{0i} + \beta_2 \text{ob/DM}_0\text{BCAA}_{1i} + \beta_3 \text{ob/DM}_1\text{BCAA}_{0i} + \beta_4 \text{ob/DM}_1\text{BCAA}_{1i} + \beta_c C_i + \beta_5 \text{ob/DM}^{*}\text{BCAA} + \varepsilon_i$$

The joint effect of BCAAs and child's sex on child risk of ASD was also investigated. The logistic regression model is represented by:

$$\ln (\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta_0 + \beta_1 \text{BCAA}_0\text{Sex}_{0i} + \beta_2 \text{BCAA}_1\text{Sex}_{0i} + \beta_3 \text{BCAA}_0\text{Sex}_{1i} + \beta_4 \text{BCAA}_1\text{Sex}_{1i} + \beta_c C_i + \varepsilon_i$$

With interaction term:

$$\ln (Pr(Y_i = ASD)/Pr(Y_i = non\text{-}ASD)) = \beta_0 + \beta_1 BCAA_0 Sex_{0i} + \beta_2 BCAA_1 Sex_{0i} + \beta_3 BCAA_0 Sex_{1i} + \beta_4 BCAA_1 Sex_{1i} + \beta_c C_i + \beta_5 BCAA^* Sex_i + \varepsilon_i$$

Further (unadjusted) joint analysis was conducted with all three risk factors: ob/DM, BCAAs, and child's sex. The logistic regression model is represented by:

$$\ln (Pr(Y_i = ASD)/Pr(Y_i = non\text{-}ASD)) = \beta_0 + \beta_1 ob/DM_0 BCAA_0 Sex_{0i} + \beta_2 ob/DM_0 BCAA_1 Sex_{0i} + \beta_3 ob/DM_1 BCAA_0 Sex_{0i} + \beta_4 ob/DM_1 BCAA_1 Sex_{0i} + \beta_5 ob/DM_0 BCAA_0 Sex_{1i} + \beta_6 ob/DM_0 BCAA_1 Sex_{1i} + \beta_7 ob/DM_1 BCAA_0 Sex_{1i} + \beta_8 ob/DM_1 BCAA_1 Sex_{1i} + \varepsilon_i$$

Analysis for Aim 2

The analysis for Aim 2 is similar to that of Aim 1. Categorical variables with four groups were created with the BCAAs and BCAA score for each maternal cholesterol measure. Because the cholesterol measurements were taken during a non-fasted state, all but HDL were not considered reliable and thus were not the focus of this aim. The logistic regression model is represented by:

$$\ln (Pr(Y_i = ASD)/Pr(Y_i = non\text{-}ASD)) = \beta_0 + \beta_1 HDL_0 BCAA_{0i} + \beta_2 HDL_0 BCAA_{1i} + \beta_3 HDL_1 BCAA_{0i} + \beta_4 HDL_1 BCAA_{1i} + \beta_c C_i + \varepsilon_i$$

With interaction term:

$$\ln (Pr(Y_i = ASD)/Pr(Y_i = non\text{-}ASD)) = \beta_0 + \beta_1 HDL_0 BCAA_{0i} + \beta_2 HDL_0 BCAA_{1i} + \beta_3 HDL_1 BCAA_{0i} + \beta_4 HDL_1 BCAA_{1i} + \beta_5 HDL^* BCAA_i + \beta_c C_i + \varepsilon_i$$

In this aim, the joint effect of HDL and child's sex on child ASD was investigated. The logistic regression model is represented by:

$$\ln (Pr(Y_i = ASD)/Pr(Y_i = non\text{-}ASD)) = \beta_0 + \beta_1 HDL_0 Sex_{0i} + \beta_2 HDL_1 Sex_{0i} + \beta_3 HDL_0 Sex_{1i} + \beta_4 HDL_1 Sex_{1i} + \beta_c C_i + \varepsilon_i$$

With interaction term:

$$\ln(\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta_0 + \beta_1 HDL_0 Sex_{0i} + \beta_2 HDL_1 Sex_{0i} + \beta_3 HDL_0 Sex_{1i} + \beta_4 HDL_1 Sex_{1i} + \beta_5 HDL^* Sex_i + \beta_c C_i + \varepsilon_i$$

Further joint analysis was conducted with all three risk factors: HDL, BCAAs, and child's sex. The logistic regression model is represented by:

$$\ln(\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta_0 + \beta_1 HDL_0 BCAA_0 Sex_{0i} + \beta_2 HDL_0 BCAA_1 Sex_{0i} + \beta_3 HDL_1 BCAA_0 Sex_{0i} + \beta_4 HDL_1 BCAA_1 Sex_{0i} + \beta_5 HDL_0 BCAA_0 Sex_{1i} + \beta_6 HDL_0 BCAA_1 Sex_{1i} + \beta_7 HDL_1 BCAA_0 Sex_{1i} + \beta_8 HDL_1 BCAA_1 Sex_{1i} + \varepsilon_i$$

Analysis for Aim 3

The analysis for Aim 3 also closely follows that of Aim 1. Logistic regression was first conducted for each MMD component on the risk of child ASD. The logistic regression model is represented by:

$$\ln(\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta 0 + \beta 1 E_i + \varepsilon_i$$

where E_i is each component for subject i.

The MMD score was also evaluated as a categorical variable for its association with child ASD risk (0, 1, 2, or 3+) and a likelihood test for trend was conducted. The logistic regression model is represented by:

$$\ln(\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta 0 + \beta 1 MMD_{0i} + \beta 1 MMD_{1i} + \beta 1 MMD_{2i} + \beta 1 MMD_{3+i} + \varepsilon_i$$

Categorical variables with four groups were created for each of the BCAAs and the BCAA score with the MMD score (binary). The logistic regression model is represented by:

$$\ln (\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta_0 + \beta_1 \text{MMD}_0\text{BCAA}_{0i} + \beta_2 \text{MMD}_0\text{BCAA}_{1i} + \beta_3 \text{MMD}_1\text{BCAA}_{0i} + \beta_4 \text{MMD}_1\text{BCAA}_{1i} + \beta_c C_i + \varepsilon_i$$

With interaction term:

$$\ln (\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta_0 + \beta_1 \text{MMD}_0\text{BCAA}_{0i} + \beta_2 \text{MMD}_0\text{BCAA}_{1i} + \beta_3 \text{MMD}_1\text{BCAA}_{0i} + \beta_4 \text{MMD}_1\text{BCAA}_{1i} + \beta_5 \text{MMD}^*\text{BCAA}_i + \beta_c C_i + \varepsilon_i$$

The unadjusted version of these models were then stratified by child's sex. Further joint analysis was conducted with all three risk factors: MMD, BCAAs, and child's sex. The logistic regression model is represented by:

$$\ln (\Pr(Y_i = \text{ASD})/\Pr(Y_i = \text{non-ASD})) = \beta_0 + \beta_1 \text{MMD}_0\text{BCAA}_0\text{Sex}_{0i} + \beta_2 \text{MMD}_0\text{BCAA}_1\text{Sex}_{0i} + \beta_3 \text{MMD}_1\text{BCAA}_0\text{Sex}_{0i} + \beta_4 \text{MMD}_1\text{BCAA}_1\text{Sex}_{0i} + \beta_5 \text{MMD}_0\text{BCAA}_0\text{Sex}_{1i} + \beta_6 \text{MMD}_0\text{BCAA}_1\text{Sex}_{1i} + \beta_7 \text{MMD}_1\text{BCAA}_0\text{Sex}_{1i} + \beta_8 \text{MMD}_1\text{BCAA}_1\text{Sex}_{1i} + \varepsilon_i$$

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CHAPTER 4 MATERNAL OBESITY/DIABETES, PLASMA BRANCHED-CHAIN AMINO ACIDS (BCAAS), AND RISK OF CHILD AUTISM SPECTRUM DISORDER: EVIDENCE OF SEX DIFFERENCE

ABSTRACT

Background:

Maternal obesity/diabetes (ob/DM) are known risk factors for child ASD. Branched-chain amino acids (BCAAs) are associated with ob/DM in adults and BCAAs were also associated with ASD in children. We examined the joint associations of maternal ob/DM and BCAAs on child ASD risk and whether the associations differed by child's.

Methods:

We analyzed 864 mother-infant pairs, a subset of the Boston Birth Cohort with pertinent data. Maternal plasma BCAAs were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in samples collected 24-72 hours postpartum. A composite BCAA score was created using factor analysis, and pre-pregnancy obesity and diabetes (ob/DM) were combined into one variable. Logistic regression was used to explore the role of BCAAs as mediators or co-factors with ob/DM and sex on child ASD risk. BCAA-ob/DM and BCAA-sex interactions were also examined.

Results:

Maternal BCAAs alone were not associated with ASD and did not mediate the path between ob/DM and ASD. In the presence of maternal ob/DM (n=202), BCAA score was significantly associated with ASD (adjusted OR 2.35, 95% CI 1.21, 4.55). Interactions were present for leucine and valine with ob/DM and for leucine and isoleucine with male sex on ASD risk. The OR was the greatest with all three risk factors – male sex, high BCAA score, and ob/DM (OR 8.72, 95% CI 3.62, 20.99). Similar patterns were found for other developmental disorders, though not as strong as for ASD.

Conclusions:

While male sex and maternal ob/DM are known risk factors of child ASD, we found elevated maternal plasma BCAAs further increased this risk. Additional studies are warranted to clarify the role of maternal BCAAs in ASD and to help elucidate mechanisms behind the 3:1 male to female ratio of ASD.

INTRODUCTION

Autism spectrum disorder (ASD) is an array of neurodevelopmental conditions characterized by deficits in social interaction and communication and by restricted or repetitive interests and behaviors.[1] The etiology of ASD is complex and poorly understood. Though highly heritable, various environmental factors are also implicated in its pathophysiology.[2] This study focuses on two major research gaps. First, maternal obesity and type II diabetes mellitus (T2DM) are important risk factors of ASD as demonstrated by us and others; however, the underlying molecular mechanisms are unclear.[3-5] Since 20-40% of mothers in developed countries enter pregnancy obese, this is a matter of great urgency.[6-8] Second, though ASD is much more common in males, with a male to female ratio of approximately 3:1, there is a lack of understanding of factors responsible for this disproportionate risk.[9]

Branched-chain amino acids (BCAAs) – leucine, isoleucine, and valine – are essential amino acids mainly found in animal-sourced foods and have important cellular signaling roles. Altered amino acids are known to be associated with obesity and diabetes.[10,11] For example, elevated circulating BCAAs are predictive of incident T2DM.[12,13] Studies also show links between BCAA and ASD with inconsistent findings.[14-16] However, the role of maternal circulating BCAAs in children ASD remains uncertain as the few metabolomics studies that exist were conducted in ASD individuals rather than in an inter-generational setting. These studies were also small in sample size and reported mostly inconsistent results. There is a lack of prospective birth cohort studies to assess the temporal relationship of maternal circulating BCAAs on child risk of developing ASD.

In light of persistently high rates of maternal obesity/diabetes and growing evidence of BCAA involvement in obesity/diabetes and ASD, we sought to investigate the joint associations of

maternal obesity/diabetes and maternal plasma BCAAs on offspring risk of ASD. Further, we aimed to clarify the role of maternal BCAAs as mediators or co-factors in the pathway from maternal obesity/diabetes to child ASD. Given the striking sex difference in ASD, we were also interested in exploring whether the aforementioned associations differ by child's. We took advantage of an intergenerational prospective cohort to illuminate the role of the prenatal metabolic environment – as assessed by both clinical measurements of maternal obesity/diabetes and maternal plasma BCAA metabolites – in the development of ASD in children.

METHODS

Participants and Data Collection Procedure

This study included 864 mother-infant pairs, a subset of the Boston Birth Cohort (BBC), an ongoing study at the Boston Medical Center (BMC). The present study includes mothers recruited from 2004 – 2015 and children prospectively followed from 2004 – 2017. Details of BBC recruitment have been previously published.[3] Both the initial and follow-up studies were approved by the Institutional Review Boards (IRB) of the Johns Hopkins Bloomberg School of Public Health and the Boston University Medical Center.

Briefly, mothers were recruited 24-72 hours post-partum, and written informed consent was obtained from the participants. Exclusion criteria included multiple births, pregnancies due to *in vitro* fertilization, babies with chromosomal abnormalities or major birth defects, and children with other developmental disorders. Only mothers with metabolite measurements and children receiving continued care at the BMC were included in this study (Supplemental Figure 4-1). Maternal and infant medical records were reviewed using standardized abstraction forms and mothers were interviewed face-to-face using a standardized questionnaire. Maternal blood was

collected at the time of enrolment and fractionated at the BMC. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to quantitatively profile maternal plasma metabolites at the Harvard-MIT Broad Institute Metabolite Profiling Laboratory.

Identification of Children with ASD

ASD was defined based on the electronic medical records (EMR) which contain clinicians' primary and secondary diagnoses using ICD-9 or ICD-10 codes from all child visits at the BMC, including primary care and subspecialty care (Supplemental Table 4-1). Based on EMR ICD codes, children who were ever diagnosed with autism (299.00), Asperger syndrome (299.80), and/or pervasive developmental disorder - not otherwise specified (299.90) constituted the ASD cases. Of note, while we did not conduct a systematic screen for ASD, the sensitivity and specificity of ASD diagnosis from the BMC is high due to its excellent autism evaluation program; all children with an ASD diagnosis were evaluated by them and they communicate regularly with the primary care pediatricians at the BMC. Children with attention deficit hyperactivity disorder (ADHD), developmental delays, or intellectual disabilities without ASD were classified as having other developmental disorders. Children without any diagnosis of ASD or other developmental disorders were classified as typically developing (TD).

Exposures

Maternal body mass index (BMI) was calculated using the pre-pregnancy weight and height obtained from the standardized questionnaire. Obesity was defined as BMI $\geq 30 kg/m^2$. Diabetes (DM) was identified by ICD-9 codes (250.00–250.93 for pre-gestational diabetes and 648.00 and 648.03 for gestational diabetes (GDM)). Because obesity and diabetes are highly correlated, they were combined into one variable and analyzed as a dichotomous variable (no obesity or DM vs

either/both obesity or DM), or a categorical variable (1. no obesity nor DM; 2. either obesity or DM; 3. both obesity and DM). Each maternal plasma BCAA was dichotomized into "below median" vs "median or above." A composite BCAA score was created using factor analysis and it was dichotomized in the same fashion.

Covariates

Maternal covariates included: age at delivery; race-ethnicity (black, white, Hispanic, or other); smoking during pregnancy ("never smoked," "ever smoked," or "continuous smoking" three months prior to pregnancy); parity (nulliparous vs multiparous), and education ("high school or less" vs "some college or more"). Child covariates included: child's sex (female vs male); and gestational age and birthweight (categorized into four groups: 1. full term (≥37 completed weeks of gestation) and non-low birthweight (non-LBW; ≥2500g); 2. full term and LBW; 3. preterm and non-LBW; 4. preterm and LBW).

Statistical Analysis

Our central hypothesis was that maternal plasma BCAA concentrations and maternal obesity and/or diabetes are jointly associated with child ASD risk. We also queried whether there is a sex difference in the association. As a first step, maternal and child characteristics for the ASD and TD groups were compared using t-tests for continuous variables and chi-squared tests for categorical variables. Missing values for categorical variables were incorporated into the largest sized group. The intensity levels of the metabolites were inverse-normally transformed to produce standardized distributions for all subsequent analyses, and any metabolite values below the limit of detection were imputed with one-half the limit of detection. A factor analysis score for BCAA (BCAA score) was calculated based on the three BCAAs using the Anderson-Rubin

Method.[17] Multinomial logistic regression modeling was conducted to explore the association between ASD and maternal BCAA metabolites as well as the BCAA score.

We further evaluated for mediation and joint effects, including interaction. Mediation analysis of BCAAs for ob/DM was conducted employing the hierarchical regression method.[18] Joint effects of BCAAs with ob/DM were also analyzed. Based on previous literature, we assigned children with maternal BCAA below median concentrations and without ob/DM as the reference group, and all three other groups (BCAAs below the median and any ob/DM, BCAAs above the median and no ob/DM, and BCAAs above the median and any ob/DM) were compared to it. We tested interactions between BCAAs and ob/DM and between BCAAs and child's sex using tests for additive and multiplicative interaction and the relative excess risk due to interaction (RERI).[19] All analyses were conducted in Stata v14.0 (Stata Corporation, College Station, TX, USA) and RStudio v1.1.423 (RStudio, Inc., Boston, MA, USA).

RESULTS

A total of 864 mother-infant pairs were included in the analysis: 89 with ASD children and 775 with TD children. Children with ASD had co-occurring, non-mutually exclusive conditions, including ADHD (n = 36) and intellectual disabilities (n = 14). On average, mothers of children diagnosed with ASD were approximately two years older than mothers of TD children ($p<0.01$) (Table 4-1). Pre-pregnancy BMI was significantly higher among mothers of ASD children than those of TD children (28.2 kg/m^2 vs 26.4 kg/m^2, $p=0.02$) as was obesity status (34.8% vs 22.1%, $p = 0.01$). DM was also more prevalent among mothers with ASD children than those with TD children, though this was only marginally significant (17.1% vs 9.9%, $p = 0.05$). More ASD children were male (31.6 percentage point difference, $p <0.0001$), born early preterm (16.1

percentage point difference, p <0.0001), and LBW (9.2 percentage point difference, p = 0.04) compared to their TD counterparts. There were no significant differences in maternal parity, race-ethnicity, education, or smoking status. Maternal and child characteristics for included and excluded participants are compared in Supplemental Table 4-2.

We reported maternal and child characteristics by level of maternal plasma BCAAs for the overall sample (Supplemental Table 4-3) and among males only (Supplemental Table 4-4). All three BCAAs were highly correlated with each other (Supplemental Figure 4-2), and there were no notable differences in the distribution of maternal BCAA score by child's sex (Supplemental Figure 4-3) nor by maternal ob/DM (Supplemental Figure 4-4).

When BCAAs were considered alone, there was no association between the BCAAs and child risk of ASD (Supplemental Table 4-5), nor did they mediate the effect of ob/DM on this risk (Supplemental Table 4-6). However, there was a synergistic effect of BCAAs and ob/DM – compared to mothers with no ob/DM and a low BCAA score, mothers with ob/DM and a high BCAA score had greater than a two-fold risk of child ASD (odds ratio (OR) 2.35, 95% CI 1.21, 4.55) (Table 4-2). Conversely, mothers with ob/DM and below median BCAA concentrations had no significant risk of bearing a child with ASD (BCAA score OR 0.96, 95% CI 0.46, 2.04). Interactions between the BCAAs and ob/DM were significant on the additive scale for leucine (p=0.04) and valine (p<0.01) but not for isoleucine or the overall BCAA score. Multiplicative interactions between valine and ob/DM on ASD risk were also present (p<0.01). Stratified odds ratios are also presented, showing differences in ORs across the strata. Sensitivity analyses were consistent with our results (Supplemental Tables 4-7 – 4-10).

We observed similar patterns for the joint effects between maternal BCAA score and child's sex on child ASD risk (Table 4-3). For the doubly-exposed category (male fetus and high BCAA

score) the BCAA score OR was 4.91, 95% CI 2.48, 9.69, while the OR for a male fetus with low BCAA score level was also significant but not as high (OR 2.61, 95% CI 1.28, 5.34). Conversely, the ORs of ASD risk for mothers carrying female fetuses were not significant, regardless of BCAA level. Further, when stratified by BCAA level, ORs for the association between child's sex and ASD outcome were notably disparate, indicating effect modification by maternal BCAA level. Tests for interaction between the BCAAs and child's sex were significant on the additive scale, with the strongest BCAA score-ASD associations found for male children (p=0.03). There was also evidence of multiplicative interaction for above median maternal isoleucine and male sex (p=0.03).

Effects of ob/DM on child risk of ASD also differed by child's sex. Compared to no ob/DM mothers with female fetuses, mothers with any ob/DM and a male fetus had a six-fold increased risk for having a child with ASD when adjusting for demographic factors (Supplemental Table 4-11). Figure 4-1 further illustrates the association of BCAA score on ASD risk, overall and by sex (a.-c.) and when stratified by ob/DM status, overall and by sex (d.-f.). These associations are also shown for individual BCAAs (Supplemental Figure 4-5). The risk of the child developing ASD was 8.7 times higher (95% CI 3.62, 20.99) in women with all three risk factors - high BCAA concentrations, ob/DM, and carrying a male fetus - combined, compared to the reference group of mothers with low BCAA concentrations, no ob/DM and carrying a female fetus (Figure 4-2).

Similar to ASD, maternal BCAAs alone were not associated with other developmental disorders (DD) (data not shown), and ob/DM was marginally associated with other DD (p=0.057, data not shown). However, a trend was also observed in analysis of the association between joint risk factors – ob/DM and BCAAs – and other DD (BCAA score OR 1.50, 95% CI 1.05, 2.13, p for interaction = 0.021) (Supplemental Table 4-12). Of note, this effect was mainly driven by

leucine, though valine also had a significant interaction (p = 0.018). As found in ASD, there was a sex difference observed for other DD as well (Supplemental Tables 4-13 - 4-14), with males at a higher risk than females (BCAA score OR 2.51 95% CI 1.59, 3.95, p for interaction = 0.033). The risk for other DD was also the highest with all three risk factors combined – male sex, maternal ob/DM, and above median maternal BCAAs – adjusted for key covariates (Supplemental Figure 4-6).

DISCUSSION

Main findings

Examined alone, maternal plasma BCAAs were not significantly associated with child ASD risk and did not mediate the risk conferred by maternal metabolic conditions. However, BCAAs did have significant joint effects with maternal ob/DM and child male sex on increasing the risk of ASD. The interactions between BCAAs and child's sex and between BCAAs and maternal ob/DM were predominantly additive, consistent with our hypothesis regarding biological mechanisms. The combination of maternal ob/DM and below median BCAA concentrations was not associated with child risk of ASD, highlighting the strong influence of BCAA concentrations in mothers with ob/DM. While child's sex and ob/DM are previously known risk factors of child ASD, our data suggest that elevated BCAA concentrations are associated with further increases in this risk. When examined altogether, the three risk factors conferred a nearly nine-fold higher risk of child ASD. These effects were also observed with other DD though they were not as strong compared to ASD.

Interpretation

Over the past several years, multiple studies have brought into focus the association between maternal ob/DM and offspring ASD.[4,5,20] While the exact biological mechanisms are not clear, metabolic conditions, like obesity and diabetes, are associated with chronic, systemic inflammation as reflected by increased concentrations of circulating cytokines and proinflammatory markers, which can pass through the placenta and adversely affect the development of the fetal brain.[21,22] Insulin also plays a role in neurodevelopment, and maternal diabetes can cause dysregulation of insulin signaling in the fetal brain.[23] BCAAs are intricately involved in the cellular mechanisms underpinning obesity and diabetes.[13,24] For example, HOMA-IR (homeostasis model assessment-insulin resistance index) is more strongly correlated with BCAAs than with fatty acids.[13] Insulin resistance leads to a decrease in BCAA catabolism and consequently an increase in circulating BCAAs. Conversely, elevated concentrations of BCAAs promote accumulation of fatty acids, which can result in insulin resistance.[24]

There are a few studies showing the link between BCAA and ASD.[14-16] While exact mechanisms underlying the role of BCAAs in ASD remain to be investigated, previous literature has raised several possibilities. The relationship between BCAAs and insulin is mediated through the mammalian target of rapamycin (mTOR) pathway. mTOR is a kinase within a vast signaling network and regulates several key cellular functions, including cellular growth and energy balance.[25] As the mTOR signaling pathway promotes synaptic protein synthesis, its dysregulation has been implicated in the etiology of ASD, in which pruning of neurons during development is inhibited.[26] Chronic activation of mTOR can inhibit the cellular uptake of glucose and lead to insulin resistance, and BCAAs, especially leucine, are known inducers of mTOR.[25,27] Though the studies on BCAAs and ASD were conducted on individuals with ASD, it

is possible that elevated BCAA concentrations in mothers may induce mTOR activity in both the mother and fetus.

Another possibility is that elevated BCAA concentrations tip the balance to increased BCAA catabolism, which can overload mitochondria and lead to defective energy metabolism.[28] This is especially true with an existing inflammatory condition such as obesity or diabetes. Disruption of beta oxidation is a precursor to mitochondrial dysfunction, a condition more prevalent in individuals with ASD and thought to be linked to its etiology.[26,29] ASD is more common among patients with propionic acidemia, a metabolic disorder marked by a deficiency in propionyl-CoA carboxylase (PCC), the enzyme responsible for the catabolism of BCAAs and other amino acids.[30] Insufficient PCC leads to disruptions in tricarboxylic acid (TCA) cycle intermediates that feed into the mitochondrial respiratory chain. Altered BCAA concentrations are also associated with other mental health disorders. For example, treatment for major depressive disorder was shown to be more effective in individuals with lower BCAA concentrations, allowing for possible prediction of response to treatment via metabolic profile analysis.[31] Furthermore, supplementation with BCAAs in mice resulted in anxiety-like behavior, a common characteristic in several neurocognitive disorders.[32] With both BCAAs and metabolic conditions linked to ASD via overlapping inflammatory pathways *in utero*, it is plausible that in combination they may contribute to a compounded risk of the disorder.

Male preponderance in ASD prevalence is well-established in the literature.[9] Our findings suggest that sexual dimorphism in ASD is influenced by maternal obesity and diabetes in conjunction with maternal plasma BCAA concentrations and thus may begin *in utero*. A recent study showed a sex-specific effect in GDM-altered maternal metabolites in second trimester amniotic fluid.[33] The authors reported while other amino acids are reduced in the amniotic fluid

milieu, leucine, along with methionine and tyrosine, was elevated with maternal GDM. Furthermore, leucine metabolites concentrations shifted more in the amniotic fluid of female fetuses than in their male counterparts. Our findings on sex differences may be explained by the theory that the female brain is better protected from inflammation *in utero* by higher concentrations of estradiol, leaving the male brain more vulnerable to inflammatory insults.[34,35] There may also be a higher threshold for genetic mutations in female fetuses before the disorder manifests.[36]

Strengths and Limitations

A particular strength of this study is its prospective, longitudinal, and intergenerational design. Additionally, we explored targeted maternal circulating metabolites specifically related to obesity and diabetes via metabolomic profiling. No other published work has reported on the association between maternal metabolites and child risk of ASD. More importantly, our study sheds light on potential maternal biomarkers in conjunction with obesity and diabetes and fetal sex on offspring risk of ASD.

As this study is the first of its kind to explore the association between maternal metabolites and child ASD, there were some limitations. The primary limitation of our study was that BCAAs were only measured at one time-point using maternal blood samples collected 24-72 hours post-delivery. Along with the physical stress associated with delivery, mothers experience changes in protein and hormone homeostasis, as well as medications during the peri-partum period. Amino acid concentrations at this time may not be reflective of a steady-state condition. Additionally, the measurement was taken in a non-fasted state. It is uncertain to what degree our observed maternal BCAA association with ASD was affected by the timing and the events peri-partum. While it is known that children with ASD were more likely to be born preterm, the associations

remained after adjusting for preterm birth. Other limitations include the lack of data as to the BCAA status of the children at the time of ASD diagnosis, whether there was a family history of ASD, as well as additional medical histories from both parents. The BBC enrolment also spanned across the transition from the American Psychiatric Association's Diagnostic and Statistical Manual fourth edition (DSM-IV) to the fifth edition (DSM-5) and from ICD-9 to ICD-10. Since the definition of ASD changed during this time, there may be inconsistencies in diagnoses between these two periods. While we adjusted for known risk factors, we cannot exclude the possibility of residual confounding.

The results from this study may not be generalizable to other populations since our study population consisted of predominantly urban, low-income minorities. However, our study helps fill a major knowledge gap since this population is mostly under-represented in ASD research. A larger sample size would have also facilitated further sex-specific and more detailed dose-response analyses. Our grouping of BCAAs was based on the exploration of the functional relationship between the maternal BCAA score and child ASD risk, as shown in Figure 4-1. Our data suggest an overall monotonic positive association, and thus our cutoff at the median was a compromise between the desire for precision and the limitation of sample size.

Of note, BCAA metabolite concentrations have mostly been reported as reduced in ASD subjects compared to TD controls.[15,16] While this may seem to contradict our findings, the maternal metabolome is not necessarily reflective of the metabolome of the child. There is no literature reporting on the outcome of offspring ASD in association with the maternal metabolome. Extrapolating from the "Barker Hypothesis" of early origins of disease, it is possible that high concentrations of BCAAs in the womb may cue the developing fetus to prepare for a similar external environment – an unlikely scenario.[37] Another possible mechanism for reduced BCAAs

in individuals with ASD could be a mutation in the branched chain ketoacid dehydrogenase kinase (BCKDK), the rate-limiting enzyme in BCAA catabolism.[38] Additionally, sensitivity to certain tastes and textures often results in poor diet quality among the ASD population and may explain the altered concentrations.[39]

Conversely, others have demonstrated a two-fold increase in concentrations of BCAA and greater mTOR activity in rabbit embryos of dams with insulin resistance and elevated circulating BCAAs compared to embryos of healthy dams. This research team concluded the BCAA composition of the blastocyst was reflective of maternal plasma and urine BCAA concentrations.[27] Another link between BCAAs and ASD is via the gut microbiome. Specific bacteria in the gut are able to synthesize BCAAs *de novo* – individuals with ASD are known to have a distinctly atypical gut microflora composition, and this could contribute to altered BCAA concentrations.[40]

This study raises more questions than it could answer, and it will hopefully stimulate more clinical and mechanistic research in this area. Future studies may consider additional time-points for metabolite measurement, including preconception and early pregnancy to avoid the influence of factors associated with the stress of delivery. It would be ideal to simultaneously examine paired maternal and child cord blood metabolome in relation to child ASD risk. The examination of plasma sampled at additional time-points during early childhood will also provide us with a greater understanding of the changes in BCAA concentrations as the disorder manifests.

The findings of this study provide important insight into the role of the BCAAs in the pathway between maternal ob/DM and child risk of ASD. Mothers with obesity or diabetes with abnormal cholesterol or other metabolic conditions may be at an even greater risk for development of child ASD when combined with elevated BCAA concentrations. However, BCAAs almost certainly

will not tell the entire story. As illustrated in Supplemental Figure 4-7, BCAA levels in the plasma are significantly associated with those of several other amino acids. Further study is required to explore the role of these additional amino acids in the pathway from maternal ob/DM to child risk of ASD. Of course, other pathways are tightly linked to amino acid metabolism as well. For example, lipid metabolites may also play an integral role in these pathways. Thus, this present study is a powerful entry point to the metabolomic exploration of ASD pathophysiology.

This study also lends further support that ASD is potentially predictable and preventable. For example, investigators from a large-scale metabolomics study recently reported dysregulation of amino acid/BCAA metabolism in close to 17% of their ASD subjects and were also able to identify metabotypes of ASD with over 90% sensitivity and specificity.[41] Thus, there is potential for similarly identifying maternal metabotypes at risk for a child with ASD, especially those with the added risk factors of ob/DM and a male fetus. This will greatly enhance our ability to detect children at high risk of ASD at the earliest possible developmental windows, when interventions may be most cost-effective.

Conclusions

In this prospective birth cohort study, we found joint associations between maternal ob/DM and elevated maternal plasma BCAAs and between child male sex and elevated maternal plasma BCAAs on child risk of ASD. These additive or synergistic effects suggest elevated maternal BCAA concentrations may further increase risk of child ASD in the setting of maternal ob/DM and/or male fetuses. Additional metabolomic studies on maternal and cord plasma BCAAs are warranted to confirm and better understand mechanistic pathways underlying these joint associations. Such new insight may inform early prediction and early intervention strategies for ASD.

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Table 4-1. Maternal and child characteristics by child autism spectrum disorder (ASD) status (Typically Developing (TD) vs. ASD) in the Boston Birth Cohort

Characteristics	Total (N=864)	TD (N=775)	ASD (N=89)	P-value[a]
Maternal age (years), mean (SD)[b]	28.21 (6.52)	27.93 (6.52)	29.95 (6.25)	0.008
Nulliparous, n (%)	379 (43.87)	344 (44.39)	35 (39.33)	0.362
Race or ethnicity, n (%)[c]				0.110
Black	611 (70.72)	557 (71.87)	54 (61.67)	
White	35 (4.05)	30 (3.87)	5 (5.62)	
Hispanic	161 (18.63)	137 (17.68)	24 (26.97)	
Other	57 (6.60)	51 (6.58)	6 (6.74)	
Maternal education, n (%)				0.986
Below college degree	741 (85.76)	666 (89.54)	75 (84.27)	
College degree or above	117 (13.54)	105 (13.55)	12 (13.48)	
Missing	6 (0.69)	4 (0.52)	2 (2.25)	
Maternal BMI, n (%)				
Mean (SD)	26.56 (6.60)	26.38 (6.41)	28.18 (7.97)	0.018
Normal weight (<25 kg/m^2)	406 (46.99)	372 (48.00)	34 (38.20)	0.009
Overweight (25 - <30 kg/m^2)	218 (25.23)	200 (25.81)	18 (20.22)	
Obese (≥30 kg/m^2)	202 (23.38)	171 (22.06)	31 (34.83)	
Missing	38 (4.40)	32 (4.13)	6 (6.74)	
Maternal Diabetes[d]				0.050
No diabetes	771 (89.24)	649 (90.14)	73 (82.95)	
Diabetes	93 (10.76)	71 (9.86)	15 (17.05)	
Maternal smoking, n (%)[e]				0.587
Never	730 (84.49)	658 (84.90)	72 (80.90)	
Quit	55 (6.37)	48 (6.19)	7 (7.87)	
Continuous	69 (7.99)	60 (7.74)	9 (10.11)	
Missing	10 (1.16)	9 (1.16)	1 (1.12)	
Child's, n (%)				<0.0001
Male	396 (45.83)	330 (42.58)	66 (74.16)	
Female	468 (54.17)	445 (57.42)	23 (25.84)	
Gestational age, n (%)				<0.0001
Term (≥37 weeks)	736 (85.19)	670 (86.45)	66 (74.16)	
Late preterm (34-36 weeks)	68 (7.87)	64 (8.26)	4 (4.49)	
Early preterm (<34 weeks)	60 (6.94)	41 (5.29)	19 (21.35)	
Birthweight				0.036
≥2,500 grams	702 (81.25)	637 (82.19)	65 (73.03)	
<2,500 grams	162 (18.75)	138 (17.81)	24 (26.97)	
Leucine (above median), n (%)	432 (50.00)	382 (49.29)	50 (56.18)	0.218
Isoleucine (above median), n (%)	432 (50.00)	386 (49.81)	46 (51.69)	0.737
Valine (above median), n (%)	432 (50.00)	376 (48.52)	52 (58.43)	0.093
BCAA score (above median), n (%)	432 (50.00)	382 (49.29)	50 (56.18)	0.171

SD, standard deviation

[a]P-values were obtained from chi-square or t-test; missing values for categorical variables were incorporated with largest sized group

[b]Maternal age at time of delivery

[c]Black includes self- reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[d]Type II diabetes mellitus and/or gestational diabetes mellitus

[e]Never smokers were defined as mothers with no history of smoking six months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of six months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting six months prior to and throughout pregnancy

Table 4-2. Association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) - joint effect with maternal obesity/diabetes (ob/DM)

	Leucine below median		Leucine above median		
Maternal obesity/DM	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM[a]
No ob/DM	26/297	1.00 (Reference)	28/317	0.97 (0.54, 1.74)	1.01 (0.58, 1.77)
Any ob/DM	13/135	0.91 (0.43, 1.93)	22/115	**2.30 (1.18, 4.67)**	**2.22 (1.06, 4.64)**
OR (95% CI) ob/DM within strata of BCAA[a]		1.11 (0.55, 2.23)		**2.44 (1.33, 4.47)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.09 (0.004, 0.18); P = 0.039** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.59 (0.98 to 6.89); P = 0.056					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM[a]
No ob/DM	28/306	1.00 (Reference)	26/308	0.85 (0.47, 1.52)	0.92 (0.52, 1.60)
Any ob/DM	15/126	1.04 (0.51, 2.14)	20/134	1.81 (0.93, 3.52)	1.42 (0.69, 2.93)
OR (95% CI) ob/DM within strata of BCAA[a]		1.34 (0.69, 2.61)		**2.09 (1.11, 3.90)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.07 (-0.02, 0.16); P = 0.135 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.04 (0.78 to 5.39); P = 0.148					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM[a]
No ob/DM	27/304	1.00 (Reference)	27/310	0.92 (0.51, 1.66)	0.98 (0.56, 1.71)
Any ob/DM	10/128	0.67 (0.30, 1.50)	25/122	**2.57 (1.34, 4.90)**	**3.04 (1.39, 6.64)**
OR (95% CI) ob/DM within strata of BCAA[a]		0.87 (0.41, 1.85)		**2.70 (1.50, 4.88)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.14 (0.05, 0.22); P = 0.003** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **4.17 (1.50 to 11.60); P = 0.006**					
	BCAA score below median		BCAA score above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM[a]
No ob/DM	26/304	1.00 (Reference)	28/310	1.05 (0.58, 2.04)	1.06 (0.61, 1.86)
Any ob/DM	13/134	0.96 (0.46, 2.04)	22/116	**2.35 (1.21, 4.55)**	**2.18 (1.04, 4.55)**

OR (95% CI) ob/DM within strata of BCAA[a]	1.15 (0.57, 2.31)	**2.36 (1.29, 4.32)**
Measure of interaction on additive scale: RERI (95% CI) = 0.09 (-0.004, 0.18); P = 0.061		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.33 (0.88 to 6.18); P = 0.090		

Note: ORs Adjusted for maternal age, race/ethnicity, education, parity, smoking status, child's, and gestational age/birthweight unless otherwise noted. [a]Stratified ORs unadjusted

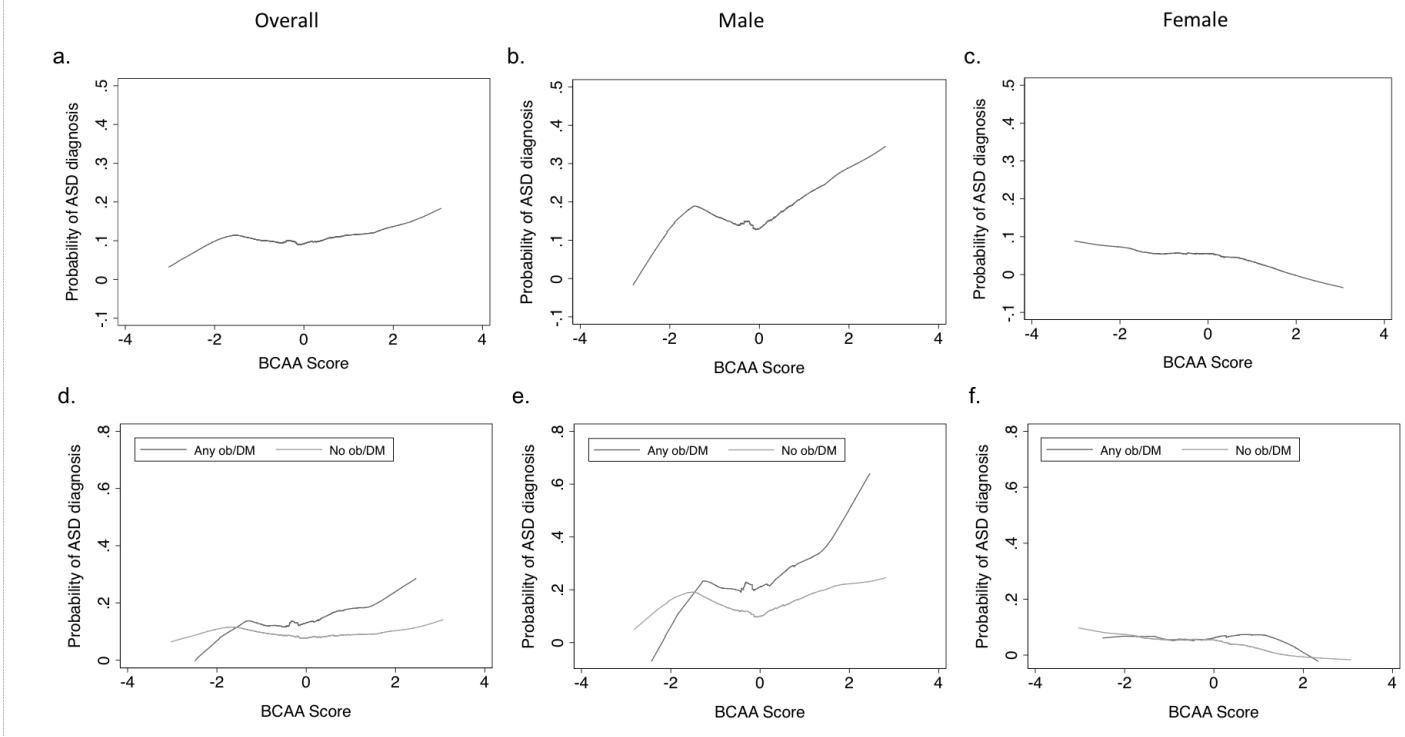
Table 4-3. Association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) - joint effect with child's

	Leucine below median		Leucine above median		
Child's sex	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of sex[a]
Female	13/240	1.00 (Reference)	10/228	0.81 (0.34, 1.93)	0.80 (0.34, 1.86)
Male	26/192	**2.77 (1.35, 5.69)**	40/204	**4.88 (2.47, 9.66)**	1.56 (0.91, 2.67)
OR (95% CI) sex within strata of BCAA[a]		**2.73 (1.36, 5.48)**		**5.32 (2.58, 10.95)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.08 (0.001, 0.16); P = 0.048**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.18 (0.77, 6.14); P = 0.142					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of sex[a]
Female	15/233	1.00 (Reference)	8/235	0.47 (0.19, 1.17)	0.51 (0.21, 1.23)
Male	28/199	**2.39 (1.21, 4.72)**	38/197	**3.73 (1.94, 7.18)**	1.46 (0.86, 2.49)
OR (95% CI) sex within strata of BCAA[a]		**2.38 (1.23, 4.60)**		**6.78 (3.08, 14.93)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.09 (0.01, 0.17); P = 0.030**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **3.29 (1.14, 9.52); P = 0.028**					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of sex[a]
Female	11/233	1.00 (Reference)	12/235	0.98 (0.41, 2.33)	1.09 (0.47, 2.51)
Male	26/199	**2.94 (1.38, 6.25)**	40/197	**5.51 (2.68, 11.32)**	1.70 (0.99, 2.91)
OR (95% CI) sex within strata of BCAA[a]		**3.03 (1.46, 6.31)**		**4.73 (2.41, 9.31)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.09 (<-0.001, 0.16); P = 0.051					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.91 (0.68, 5.35); P = 0.219					
	BCAA score below median		BCAA score above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of sex[a]
Female	13/240	1.00 (Reference)	10/228	0.76 (0.32, 1.81)	0.80 (0.34, 1.86)
Male	26/198	**2.61 (1.28, 5.34)**	40/198	**4.91 (2.48, 9.69)**	1.67 (0.97, 2.87)

OR (95% CI) sex within strata of BCAA[a]	**2.64 (1.32, 5.29)**	**5.52 (2.68, 11.37)**
Measure of interaction on additive scale: RERI (95% CI) = **0.09 (0.01, 0.17); P = 0.025**		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.48 (0.88, 7.03); P = 0.087		

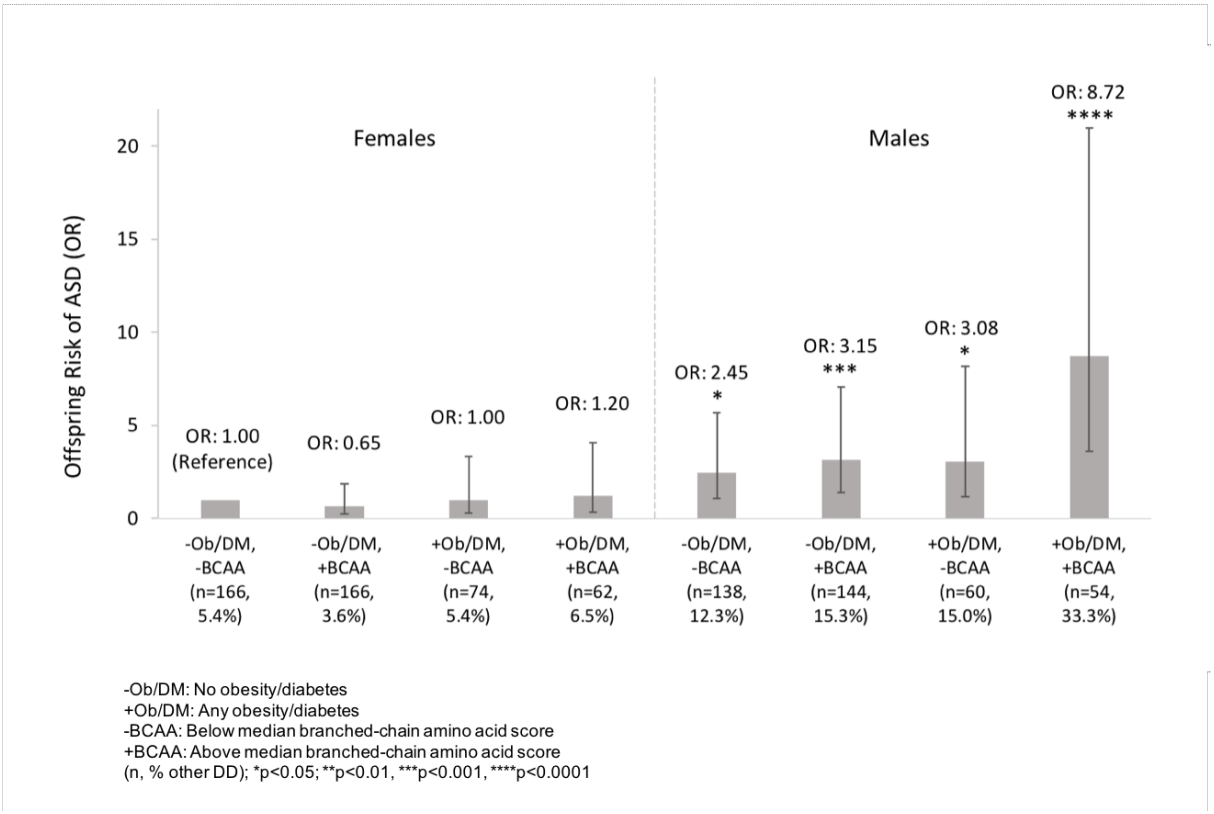
Note: ORs adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, and gestational age/birthweight unless otherwise noted. [a]Stratified ORs unadjusted

Figure 4-1. Association of maternal plasma branched-chain amino acid (BCAA) score with risk of child autism spectrum disorder (ASD) and by obesity/diabetes (ob/DM) status, overall and by sex



a. association of maternal plasma BCAA score with risk of ASD in children in the overall sample, b. association of maternal plasma BCAA score with risk of ASD in male children, c. association of maternal plasma BCAA score with risk of ASD in female children, d. association of maternal plasma BCAA score with risk of ASD in children by obesity/DM status in the overall sample, e. association of maternal plasma BCAA score with risk of ASD in male children by obesity/DM status, f. association of maternal plasma BCAA score with risk of ASD in female children by obesity/DM status.

Figure 4-2. Joint association of maternal plasma branched-chain amino acid (BCAA) score, obesity/diabetes status, and child's sex on risk of child autism spectrum disorder (ASD)



Supplemental Table 4-1. List of ICD-9 and ICD-10 codes for the diagnosis of each developmental disorder

Developmental disorder	ICD-9 codes	ICD-10 codes
ASD	299.0, 299.00, 299.01, 299.8, 299.80, 299.81, 299.9, 299.90, 299.91	F84.0, F84.8, F84.9
ADHD	314.0, 314.00, 314.01, 314.1, 314.2, 314.8, 314.9	F90, F90.0, F90.1, F90.2, F90.8, F90.9
Developmental delays	315.0, 315.9	F81.0, R48.0, F81.81, F81.2, F81.89, F80.1, F80.2, H93.25, F80.4, F80.81, F80.0, F80.82, F80.89, F82, F88, F81.9, F89
Intellectual disabilities	317 – 319	F70, F71, F72, F73, F78, F79

Supplemental Table 4-2. Maternal and child characteristics between participants excluded and included in the analysis

Characteristics	Total, N (%)	Excluded, N (%)	Included, N (%)	P-value[a]
Total	3138 (100.00)	2274 (72.47)	864 (27.53)	
Maternal age (years), mean (SD)[b]	28.64 (6.50)	28.64 (6.48)	28.21 (6.51)	0.094
Nulliparous, n (%)	1337 (42.61)	958 (42.13)	379 (43.87)	0.379
Race or ethnicity, n (%)[c]				<0.0001
Black	1998 (63.67)	1387 (60.99)	611 (70.72)	
White	227 (7.23)	192 (8.44)	35 (4.05)	
Hispanic	701 (22.34)	540 (23.75)	161 (18.63)	
Other	212 (6.76)	155 (6.82)	57 (6.60)	
Maternal education, n (%)				0.973
Below college degree	2690 (85.72)	1949 (85.71)	741 (85.76)	
College degree or above	426 (13.58)	309 (13.59)	117 (13.54)	
Missing	22 (0.70)	16 (0.70)	6 (0.69)	
Maternal BMI, n (%)				
Mean (SD)	26.58 (6.65)	26.59 (6.67)	26.56 (6.60)	0.918
Normal weight (<25)	1452 (46.27)	1046 (46.00)	406 (46.99)	0.842
Overweight (25 - <30)	813 (25.90)	595 (26.17)	218 (25.23)	
Obese (≥30)	703 (22.40)	501 (22.03)	202 (23.38)	
Missing	170 (5.42)	132 (5.80)	38 (4.40)	
Maternal Diabetes[d]				0.099
No diabetes	2751 (87.67)	1980 (89.68)	771 (89.24)	
Diabetes	387 (12.33)	294 (12.93)	93 (10.76)	
Maternal smoking, n (%)[e]				
Never	2542 (81.01)	1812 (79.68)	730 (84.49)	0.001
Quit	241 (7.68)	186 (8.18)	55 (6.37)	
Continuous	336 (10.71)	267 (11.74)	69 (7.99)	
Missing	19 (0.61)	9 (0.40)	10 (1.16)	
Child's, n (%)				0.001
Male	1583 (50.45)	1187 (52.20)	396 (45.83)	
Female	1555 (49.55)	1087 (47.80)	468 (54.17)	
Gestational age, n (%)				0.001
Term (≥37 weeks)	2448 (78.01)	1712 (75.29)	736 (85.19)	
Late preterm (34-36 weeks)	306 (9.75)	238 (10.47)	68 (7.87)	
Early preterm (<34 weeks)	384 (12.24)	324 (14.25)	60 (6.94)	
Birthweight				<0.0001
≥2,500 grams	2275 (72.50)	1573 (69.17)	702 (81.25)	
<2,500 grams	863 (27.50)	701 (30.83)	162 (18.75)	

SD, standard deviation

[a]P-values were obtained from chi-square or t-tests; missing values for categorical variables incorporated with largest sized group

[b]Maternal age at time of delivery

[c]Black includes self-reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[d]Type II diabetes mellitus and/or gestational diabetes mellitus

[e]Never smokers were defined as mothers with no history of smoking six months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of six months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting six months prior to and throughout pregnancy

Supplemental Table 4-3. Maternal and child characteristics by maternal plasma branched-chained amino acid (BCAA) score in the Boston Birth Cohort

Maternal and child characteristics	Low score (N=438)[a]	High score (N=426)	P-value[b]
ASD, n (%)	39 (8.90)	50 (11.74)	0.171
Maternal age (years), mean (SD)[c]	28.16 (6.67)	28.25 (6.37)	0.833
Nulliparous, n (%)	197 (44.98)	182 (42.72)	0.504
Race or ethnicity, n (%)[d]			0.112
Black	296 (67.58)	315 (73.94)	
White	16 (3.65)	19 (4.46)	
Hispanic	93 (21.23)	68 (15.96)	
Other	33 (7.53)	24 (5.63)	
Maternal education, n (%)			0.737
Below college degree	373 (85.16)	368 (86.38)	
College degree or above	61 (13.93)	56 (13.15)	
Missing (n=6)	4 (0.91)	2 (0.47)	
Maternal BMI, n (%)			
Mean (SD)	26.68 (6.76)	26.44 (6.44)	0.596
Normal weight (<25 kg/m^2)	205 (46.80)	201 (47.18)	0.933
Overweight (25 - <30 kg/m^2)	109 (24.89)	109 (25.59)	
Obese (≥30 kg/m^2)	108 (24.66)	94 (22.07)	
Missing (n=38)	16 (3.65)	22 (5.16)	
Maternal Diabetes			0.397
No diabetes	387 (88.36)	384 (90.14)	
Diabetes	51 (11.64)	42 (9.86)	
Maternal smoking, n (%)[e]			0.839
Never	366 (83.56)	364 (85.45)	
Quit	30 (6.85)	25 (5.87)	
Continuous	35 (7.99)	34 (7.98)	
Missing (n=10)	7 (1.60)	3 (0.70)	
Child's, n (%)			0.707
Male	198 (45.21)	198 (46.48)	
Female	240 (54.79)	228 (53.52)	
Gestational age, n (%)			0.068
Term (≥37 weeks)	383 (87.44)	353 (82.86)	
Late preterm (34, 36 weeks)	33 (7.53)	35 (8.22)	
Early preterm (<34 weeks)	22 (5.02)	38 (8.92)	
Birthweight			0.586
≥2,500 grams	359 (81.96)	343 (80.52)	
<2,500 grams	79 (18.04)	83 (19.48)	

SD, standard deviation

[a]Score derived from factor analysis as the average score of the three BCAAs (leucine, isoleucine, and valine); low is below median and high is above median

[b]P-values were obtained from chi-square or t-test; missing values for categorical variables incorporated with largest sized group

[c]Maternal age at time of delivery

[d]Black includes self, reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[e]Never smokers were defined as mothers with no history of smoking six months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of six months prior to conception and delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting six months prior to and throughout pregnancy

Supplemental Table 4-4. Maternal and child characteristics by maternal plasma branched-chain amino acid (BCAA) score in the Boston Birth Cohort among males

Maternal and child characteristics	Low score (N=198)[a]	High score (N=198)	P-value[b]
ASD, n (%)	26 (13.13)	40 (20.20)	0.059
Maternal age (years), mean (SD)[c]	27.98 (6.77)	28.25 (6.48)	0.685
Nulliparous, n (%)	94 (47.47)	87 (43.94)	0.480
Race or ethnicity, n (%)[d]			0.270
Black	130 (65.66)	146 (73.74)	
White	8 (4.04)	8 (4.04)	
Hispanic	41 (20.71)	33 (16.67)	
Other	19 (9.60)	11 (5.56)	
Maternal education, n (%)			0.884
Below college degree	169 (85.35)	170 (85.86)	
College degree or above	27 (13.64)	28 (14.14)	
Missing (n=2)	2 (1.01)	0 (0.00)	
Maternal BMI, n (%)			
Mean (SD)	26.09 (6.20)	26.25 (6.20)	0.802
Normal weight (<25)	95 (47.98)	92 (46.46)	0.897
Overweight (25 - <30)	55 (27.78)	54 (27.27)	
Obese (≥30)	42 (21.21)	41 (20.71)	
Missing (n=17)	6 (3.03)	11 (5.56)	
Maternal Diabetes			0.294
No diabetes	169 (85.35)	176 (88.89)	
Diabetes	29 (14.65)	22 (11.11)	
Maternal smoking, n (%)[e]			0.711
Never	167 (84.34)	172 (86.87)	
Quit	14 (7.07)	13 (6.57)	
Continuous	16 (8.08)	12 (6.06)	
Missing (n=2)	1 (0.51)	1 (0.51)	
Gestational age, n (%)			0.581
Term (≥37 weeks)	171 (86.36)	168 (84.85)	
Late preterm (34 - 36 weeks)	16 (8.08)	14 (7.07)	
Early preterm (<34 weeks)	11 (5.56)	16 (8.08)	
Birthweight			0.292
≥2,500 grams	159 (80.30)	343 (80.52)	
<2,500 grams	39 (19.70)	83 (19.48)	

SD, standard deviation

[a]Score derived from factor analysis as the average score of the three BCAAs (leucine, isoleucine, and valine); low is below median and high is above median

[b]P-values were obtained from chi-square or t-test; missing values for categorical variables incorporated with largest sized group

[c]Maternal age at time of delivery

[d]Black includes self, reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[e]Never smokers were defined as mothers with no history of smoking six months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of six months prior to conception and delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting six months prior to and throughout pregnancy

Supplemental Table 4-5. Association between maternal plasma branched-chain amino acid (BCAA) score and risk of child autism spectrum disorder (ASD), where BCAA score was analyzed as linear, in tertiles, and binary

BCAA	**ASD OR (95% CI)[a]**	**ASD OR (95% CI)[b]**
Leucine		
Linear	1.11 (0.89, 1.38)	1.12 (0.88, 1.42)
Tertiles		
T1 (N= 288)	Reference	Reference
T2 (N= 288)	1.04 (0.59, 1.83)	0.89 (0.49, 1.61)
T3 (N= 288)	1.44 (0.84, 2.45)	1.38 (0.78, 2.43)
Binary[c]	1.32 (0.85, 2.05)	1.40 (0.87, 2.24)
Isoleucine		
Linear	1.13 (0.90, 1.41)	1.14 (0.89, 1.44)
Tertiles		
T1 (N= 288)	Reference	Reference
T2 (N= 288)	0.78 (0.45, 1.37)	0.68 (0.37, 1.23)
T3 (N= 288)	1.19 (0.71, 2.00)	1.16 (0.67, 2.01)
Binary[c]	1.08 (0.70, 1.67)	1.11 (0.69, 1.76)
Valine		
Linear	1.08 (0.87, 1.35)	1.10 (0.87, 1.41)
Tertiles		
T1 (N= 288)	Reference	Reference
T2 (N= 288)	0.85 (0.48, 1.49)	0.91 (0.50, 1.64)
T3 (N= 288)	1.24 (0.73, 2.08)	1.27 (0.73, 2.20)
Binary[c]	1.46 (0.94, 2.28)	1.55 (0.97, 2.49)
BCAA score		
Linear	1.11 (0.89, 1.39)	1.12 (0.88, 1.43)
Tertiles		
T1 (N= 288)	Reference	Reference
T2 (N= 288)	0.96 (0.55, 1.69)	0.82 (0.45, 1.49)
T3 (N= 288)	1.38 (0.81, 2.34)	1.31 (0.75, 2.29)
Binary[c]	1.36 (0.87, 2.12)	1.44 (0.90, 2.30)

[a]Unadjusted analysis
[b]Adjusted for maternal age, race, parity, education, smoking, obesity/DM status, child's, and birthweight/gestational age
[c]Cut-off at the median

Supplemental Table 4-6. Mediation analysis – maternal plasma branched-chain amino acid (BCAA) score as a mediator in the relationship between maternal obesity/diabetes (ob/DM) and risk of child ASD

Total N=720; ASD=88	Total Effect, OR (95% CI)
Model 1	
No ob/DM	Reference
Any ob/DM	1.69 (1.07, 2.66)
Model 2	
No ob/DM	Reference
Any ob/DM	1.69 (1.07, 2.67)
Model 3	
No ob/DM	Reference
Any ob/DM	1.51 (0.93, 2.47)
Model 4	
No ob/DM	Reference
Any ob/DM	1.53 (0.94, 2.50)

Model 1 Unadjusted
Model 2 Adjusted for continuous BCAA score variable
Model 3 Adjusted for maternal age, race, parity, education, smoking, child's, and gestational age/birthweight
Model 4 Adjusted for maternal age, race, parity, education, smoking, child's, gestational age/birthweight, and continuous BCAA score variable

Supplemental Table 4-7. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of child ASD - joint effect with maternal obesity/diabetes (ob/DM)

	Leucine below median		Leucine above median		
Maternal ob/DM	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	26/297	1.00 (Reference)	28/317	1.01 (0.58, 1.77)	1.01 (0.58, 1.77)
Any ob/DM	13/135	1.11 (0.55, 2.23)	22/115	**2.47 (1.33, 4.56)**	**2.22 (1.06, 4.64)**
OR (95% CI) ob/DM within strata of BCAA		1.11 (0.55, 2.23)		**2.44 (1.33, 4.47)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.09 (-0.004, 0.19); P = 0.060					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.59 (0.98 to 6.89); P = 0.056					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	28/306	1.00 (Reference)	26/308	0.92 (0.52, 1.60)	0.92 (0.52, 1.60)
Any ob/DM	15/126	1.34 (0.69, 2.61)	20/134	**1.91 (1.03, 3.54)**	1.42 (0.69, 2.93)
OR (95% CI) ob/DM within strata of BCAA		1.34 (0.69, 2.61)		**2.09 (1.11, 3.90)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.05 (-0.05, 0.15); P = 0.319					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.04 (0.78 to 5.39); P = 0.148					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	27/304	1.00 (Reference)	27/310	0.98 (0.56, 1.71)	0.98 (0.56, 1.71)
Any ob/DM	10/128	0.87 (0.41, 1.85)	25/122	**2.64 (1.46, 4.78)**	**3.04 (1.39, 6.64)**
OR (95% CI) ob/DM within strata of BCAA		0.87 (0.41, 1.85)		**2.70 (1.50, 4.88)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.13 (0.03, 0.22); P = 0.009**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **4.17 (1.50 to 11.60); P = 0.006**					
	BCAA score below median		BCAA score above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	26/304	1.00 (Reference)	28/310	1.06 (0.61, 1.86)	1.06 (0.61, 1.86)
Any ob/DM	13/134	1.15 (0.57, 2.31)	22/116	**2.50 (1.35, 4.62)**	**2.18 (1.04, 4.55)**

OR (95% CI) ob/DM within strata of BCAA	1.15 (0.57, 2.31)	**2.36 (1.29, 4.32)**
Measure of interaction on additive scale: RERI (95% CI) = 0.09 (-0.01, 0.19); P = 0.079		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.33 (0.88 to 6.18); P = 0.090		

Supplemental Table 4-8. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) - joint effect with maternal obesity/diabetes (ob/DM), where the analyses were limited to males

	Leucine below median		Leucine above median		
Maternal ob/DM	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	17/132	1.00 (Reference)	22/150	1.16 (0.59, 2.30)	1.16 (0.59, 2.30)
Any ob/DM	9/60	1.19 (0.50, 2.86)	18/54	**3.38 (1.58, 7.24)**	**2.83 (1.14, 7.02)**
OR (95% CI) ob/DM within strata of BCAA		1.19 (0.50, 2.86)		**2.91 (1.41, 6.00)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.17 (-0.01, 0.34); P = 0.063					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.44 (0.78, 7.58); P = 0.124					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	17/139	1.00 (Reference)	22/143	1.30 (0.66, 2.58)	1.30 (0.66, 2.58)
Any ob/DM	11/60	1.61 (0.70, 3.69)	16/54	**3.02 (1.39, 6.55)**	1.88 (0.78, 4.51)
OR (95% CI) ob/DM within strata of BCAA		1.61 (0.70, 3.69)		**2.32 (1.10, 4.85)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.08 (-0.09, 0.26); P = 0.364					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.44 (0.47, 4.36); P = 0.522					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	18/141	1.00 (Reference)	21/141	1.20 (0.61, 2.36)	1.20 (0.61, 2.36)
Any ob/DM	8/58	1.09 (0.47, 2.68)	19/56	**3.51 (1.67, 7.37)**	**3.21 (1.27, 8.13)**
OR (95% CI) ob/DM within strata of BCAA		1.09 (0.47, 2.68)		**2.93 (1.43, 6.04)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.18 (0.01, 0.35); P = 0.041**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.68 (0.85, 8.48); P = 0.139					
	BCAA score below median		BCAA score above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	17/138	1.00 (Reference)	22/144	1.28 (0.68, 2.54)	1.28 (0.65, 2.54)
Any ob/DM	9/60	1.26 (0.53, 3.00)	18/54	**3.56 (1.35, 1.66)**	**2.83 (1.14, 7.02)**

OR (95% CI) ob/DM within strata of BCAA	1.26 (0.53, 3.00)	**2.78 (1.34, 5.73)**
Measure of interaction on additive scale: RERI (95% CI) = 0.15 (-0.02, 0.33); P = 0.084		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.21 (0.71, 6.86); P = 0.171		

Supplemental Table 4-9. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) - joint effect with maternal obesity/diabetes (ob/DM), where the analyses were limited to females

	Leucine below median		Leucine above median		
Maternal ob/DM	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	17/132	1.00 (Reference)	22/150	0.65 (0.22, 1.86)	0.65 (0.22, 1.86)
Any ob/DM	9/60	0.98 (0.29, 3.28)	18/54	1.22 (0.36, 4.10)	1.25 (0.30, 5.20)
OR (95% CI) ob/DM within strata of BCAA		0.98 (0.29, 3.28)		1.88 (0.51, 6.91)	
Measure of interaction on additive scale: RERI (95% CI) = 0.03 (-0.06, 0.12); P = 0.510					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.93 (0.33, 11.40); P = 0.171					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	17/139	1.00 (Reference)	22/143	0.35 (0.11, 1.13)	0.35 (0.11, 1.13)
Any ob/DM	11/60	0.91 (0.28, 2.98)	16/54	0.86 (0.26, 2.80)	0.94 (0.23, 3.92)
OR (95% CI) ob/DM within strata of BCAA		0.91 (0.28, 2.98)		2.44 (0.59, 10.04)	
Measure of interaction on additive scale: RERI (95% CI) = 0.04 (-0.05, 0.13); P = 0.410					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.67 (0.42, 16.84); P = 0.297					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	18/141	1.00 (Reference)	21/141	0.63 (0.22, 1.81)	0.63 (0.22, 1.81)
Any ob/DM	8/58	0.50 (0.11, 2.39)	19/56	1.71 (0.58, 5.01)	3.40 (0.66, 17.48)
OR (95% CI) ob/DM within strata of BCAA		0.50 (0.11, 2.39)		2.72 (0.84, 8.75)	
Measure of interaction on additive scale: RERI (95% CI) = 0.08 (-0.01, 0.17); P = 0.078					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 5.40 (0.77, 37.89); P = 0.090					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	17/138	1.00 (Reference)	22/144	0.65 (0.23, 1.88)	0.65 (0.23, 1.88)
Any ob/DM	9/60	1.00 (0.30, 3.35)	18/54	1.20 (0.36, 4.06)	1.21 (0.29, 5.04)

OR (95% CI) ob/DM within strata of BCAA	1.00 (0.30, 3.35)	1.84 (0.50, 6.75)
Measure of interaction on additive scale: RERI (95% CI) = 0.03 (-0.06, 0.12); P = 0.541		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.84 (0.31, 10.91); P = 0.499		

Supplemental Table 4-10. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) - joint effect with maternal obesity/diabetes (ob/DM) (neither, either, or both)

	Leucine below median		Leucine above median	
Maternal ob/DM	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)
Neither ob/DM	26/297	1.00 (Reference)	28/317	1.01 (0.58, 1.77)
Either ob/DM	8/109	0.83 (0.36, 1.88)	16/96	2.08 (1.07, 4.08)
Both ob/DM	5/26	2.48 (0.86, 7.13)	6/19	**4.81 (1.69, 13.72)**
	Isoleucine below median		Isoleucine above median	
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)
Neither ob/DM	28/306	1.00 (Reference)	26/308	0.92 (0.52, 1.60)
Either ob/DM	10/104	1.06 (0.49, 2.26)	14/101	1.60 (0.81, 3.17)
Both ob/DM	5/22	**2.92 (1.00, 8.51)**	6/23	**3.50 (1.28, 9.61)**
	Valine below median		Valine above median	
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)
Neither ob/DM	27/304	1.00 (Reference)	27/310	0.98 (0.56, 1.71)
Either ob/DM	6/104	0.63 (0.25, 1.57)	18/101	**2.22 (1.17, 4.24)**
Both ob/DM	4/24	2.05 (0.65, 6.44)	7/21	**5.13 (1.91, 13.80)**
	BCAA score below median		BCAA score above median	
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)
Neither ob/DM	26/304	1.00 (Reference)	28/310	1.06 (0.61, 1.86)
Either ob/DM	8/109	0.85 (0.37, 1.93)	16/96	**2.14 (1.09, 4.18)**
Both ob/DM	5/25	2.67 (0.93, 7.71)	6/20	**4.58 (1.62, 12.93)**

Supplemental Table 4-11. Association between child's sex and risk of child autism spectrum disorder (ASD) – joint effect with maternal obesity/diabetes (ob/DM)

	No maternal ob/DM		Any maternal ob/DM		
Child's sex	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) ob/DM within strata of sex*
Female	13/240	1.00 (Reference)	10/228	1.15 (0.47, 2.85)	1.32 (0.55, 3.19)
Male	26/198	**3.54 (1.89, 6.65)**	40/198	**6.01 (3.00, 12.03)**	**1.93 (1.12, 3.35)**
OR (95% CI) sex within strata of ob/DM*		**3.39 (1.83, 6.30)**		**4.97 (2.16, 11.44)**	

Measure of interaction on additive scale: RERI (95% CI) = 0.07 (-0.03, 0.16); P = 0.165
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.47 (0.511, 4.26); P = 0.475

Supplemental Table 4-12. Association of maternal plasma branched-chain amino acids (BCAAs) and risk of other child developmental disorders (DD) - joint effect with maternal obesity/diabetes (ob/DM)

	Leucine score below median		Leucine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	188/464	1.00 (Reference)	180/464	0.98 (0.75, 1.29)	0.99 (0.75, 1.31)
Any ob/DM	80/203	0.92 (0.65, 1.31)	98/190	**1.53 (1.07, 2.19)**	**1.86 (1.41, 2.46)**
OR (95% CI) ob/DM within strata of BCAA		0.89 (0.62, 1.28)		**1.56 (1.09, 2.23)**	
Measure of interaction on additive scale: **RERI (95% CI) = 0.14 (0.02, 0.26); P = 0.019**					
Measure of interaction on multiplicative scale: **ratio of ORs (95% CI) = 1.70 (1.03 to 2.79); P = 0.038**					
	Isoleucine score below median		Isoleucine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	195/472	1.00 (Reference)	173/456	0.90 (0.68, 1.19)	0.90 (0.68, 1.19)
Any ob/DM	80/191	0.96 (0.67, 1.37)	98/202	1.32 (0.93, 1.87)	1.37 (0.90, 2.10)
OR (95% CI) ob/DM within strata of BCAA		0.94 (0.65, 1.36)		**1.46 (1.03, 2.06)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.10 (-0.02, 0.22); P = 0.093					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.53 (0.93 to 2.52); P = 0.095					
	Valine score below median		Valine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	197/472	1.00 (Reference)	171/456	0.87 (0.66, 1.15)	0.88 (0.66, 1.16)
Any ob/DM	80/198	0.91 (0.64, 1.29)	98/195	1.36 (0.95, 1.94)	1.49 (0.98, 2.28)
OR (95% CI) ob/DM within strata of BCAA		0.90 (0.63, 1.30)		**1.55 (1.09, 2.21)**	
Measure of interaction on additive scale: **RERI (95% CI) = 0.14 (0.02, 0.26); P = 0.018**					
Measure of interaction on multiplicative scale: **ratio of ORs (95% CI) = 1.72 (1.04 to 2.83); P = 0.034**					
	BCAA score below median		BCAA score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	190/468	1.00 (Reference)	178/460	0.97 (0.74, 1.28)	0.98 (0.74, 1.29)
Any ob/DM	79/200	0.92 (0.65, 1.31)	99/193	**1.50 (1.05, 2.13)**	**1.60 (1.05, 2.45)**

OR (95% CI) ob/DM within strata of BCAA	0.89 (0.61, 1.28)	**2.36 (1.29, 4.32)**

Measure of interaction on additive scale: **RERI (95% CI) = 0.14 (0.02, 0.25); P = 0.021**
Measure of interaction on multiplicative scale: **ratio of ORs (95% CI) = 1.67 (1.02 to 2.75); P = 0.043**

Note: ORs Adjusted for maternal age, race/ethnicity, education, parity, smoking status, child's, and gestational age/birthweight

Supplemental Table 4-13. Association of maternal plasma branched-chain amino acids (BCAAs) and risk of other child developmental disorders (DD) - joint effect with maternal obesity/diabetes (ob/DM), among male children

	Leucine score below median		Leucine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	118/234	1.00 (Reference)	98/225	1.35 (0.99, 1.83)	0.86 (0.58, 1.27)
Any ob/DM	48/99	1.52 (0.99, 2.34)	56/98	**2.49 (1.58, 3.91)**	1.47 (0.79, 2.34)
OR (95% CI) ob/DM within strata of BCAA		0.89 (0.54, 1.47)		**1.85 (1.10, 3.11)**	
Measure of interaction on additive scale: **RERI (95% CI) = 0.19 (0.03, 0.36); P = 0.024** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.98 (0.98 to 4.03); P = 0.059					
	Isoleucine score below median		Isoleucine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	126/247	1.00 (Reference)	90/212	1.25 (0.92, 1.71)	0.76 (0.51, 1.13)
Any ob/DM	51/100	**1.63 (1.06, 2.50)**	53/91	**2.22 (1.42, 3.49)**	1.28 (0.69, 2.37)
OR (95% CI) ob/DM within strata of BCAA		0.93 (0.56, 1.53)		**1.75 (1.04, 2.93)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.16 (-0.01, 0.33); P = 0.065 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.84 (0.91 to 3.74); P = 0.091					
	Valine score below median		Valine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	128/251	1.00 (Reference)	88/208	1.25 (0.91, 1.70)	0.76 (0.51, 1.13)
Any ob/DM	53/103	**1.72 (1.13, 2.63)**	51/88	**2.09 (1.32, 3.32)**	1.11 (0.60, 2.05)
OR (95% CI) ob/DM within strata of BCAA		0.97 (0.60, 1.59)		1.65 (0.97, 2.81)	
Measure of interaction on additive scale: RERI (95% CI) = 0.15 (-0.02, 0.32); P = 0.077 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.65 (0.81 to 3.36); P = 0.164					
	BCAA score below median		BCAA score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	120/241	1.00 (Reference)	96/218	**1.38 (1.01, 1.87)**	0.89 (0.60, 1.32)
Any ob/DM	48/99	1.51 (0.98, 2.33)	56/92	**2.51 (1.59, 3.95)**	1.48 (0.79, 2.74)

OR (95% CI) ob/DM within strata of BCAA	0.91 (0.55, 1.49)	**1.83 (1.08, 3.07)**

Measure of interaction on additive scale: **RERI (95% CI) = 0.18 (0.01, 0.35); P = 0.033**
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.93 (0.95 to 3.92); P = 0.069

Note: ORs Adjusted for maternal age, race/ethnicity, education, parity, smoking status, and gestational age/birthweight

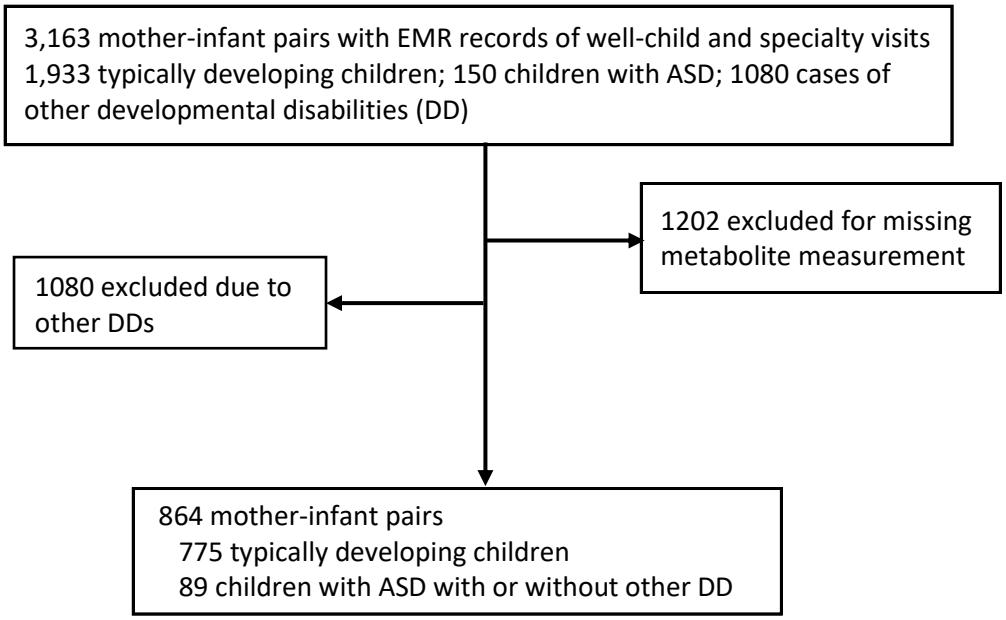
Supplemental Table 4-14. Association of maternal plasma branched-chain amino acids (BCAAs) and risk of other child developmental disorders (DD) - joint effect with maternal obesity/diabetes (ob/DM), among female children

	Leucine score below median		Leucine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	70/230	1.00 (Reference)	83/239	**0.66 (0.50, 0.90)**	1.21 (0.81, 1.82)
Any ob/DM	32/104	**0.51 (0.32, 0.80)**	42/98	0.87 (0.56, 1.34)	1.67 (0.91, 3.08)
OR (95% CI) ob/DM within strata of BCAA		0.95 (0.55, 1.63)		1.30 (0.78, 2.15)	
Measure of interaction on additive scale: RERI (95% CI) = 0.08 (-0.07, 0.24); P = 0.305 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.42 (0.69 to 2.90); P = 0.342					
	Isoleucine score below median		Isoleucine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	69/225	1.00 (Reference)	83/244	**0.63 (0.46, 0.85)**	1.12 (0.75, 1.67)
Any ob/DM	29/91	**0.54 (0.33, 0.86)**	45/111	0.77 (0.50, 1.16)	1.33 (0.71, 2.49)
OR (95% CI) ob/DM within strata of BCAA		0.99 (0.56, 1.75)		1.21 (0.74, 1.98)	
Measure of interaction on additive scale: RERI (95% CI) = 0.05 (-0.10, 0.21); P = 0.507 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.28 (0.62 to 2.63); P = 0.501					
	Valine score below median		Valine score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	69/221	1.00 (Reference)	83/248	**0.60 (0.44, 0.82)**	1.04 (0.70, 1.56)
Any ob/DM	27/95	**0.45 (0.28, 0.73)**	47/107	0.87 (0.57, 1.32)	**1.93 (1.04, 3.58)**
OR (95% CI) ob/DM within strata of BCAA		0.83 (0.46, 1.49)		1.45 (0.89, 2.36)	
Measure of interaction on additive scale: RERI (95% CI) = 0.13 (-0.02, 0.29); P = 0.095 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.83 (0.88 to 3.79); P = 0.104					
	BCAA score below median		BCAA score above median		
	N other DD/ Total	OR (95% CI)	N other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM
No ob/DM	70/227	1.00 (Reference)	82/242	**0.63 (0.47, 0.86)**	1.14 (0.76, 1.71)
Any ob/DM	31/101	**0.51 (0.33, 0.81)**	43/101	0.83 (0.54, 1.28)	1.62 (0.88, 2.98)

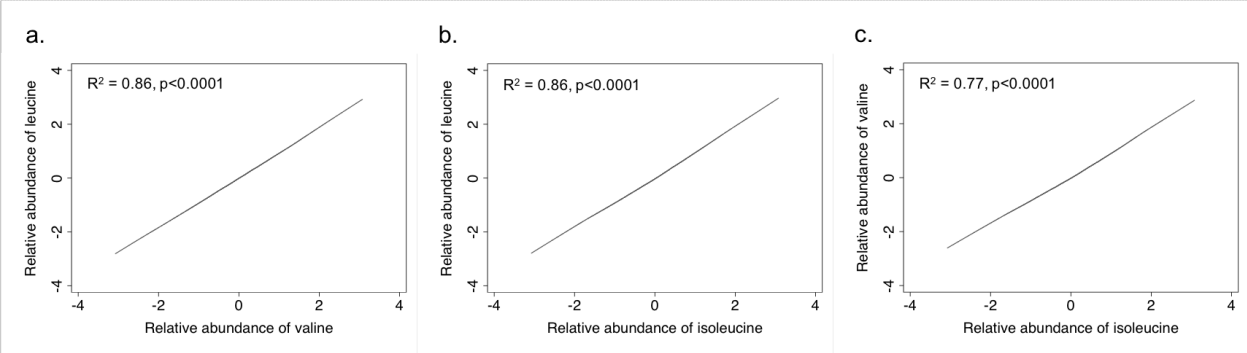
OR (95% CI) ob/DM within strata of BCAA	0.93 (0.53, 1.63)	1.30 (0.80, 2.18)
Measure of interaction on additive scale: RERI (95% CI) = 0.09 (-0.07, 0.25); P = 0.269		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.41 (0.69 to 2.88); P = 0.347		

Note: ORs Adjusted for maternal age, race/ethnicity, education, parity, smoking status, and gestational age/birthweight

Supplemental Figure 4-1. Flowchart of study sample included and excluded in the analyses

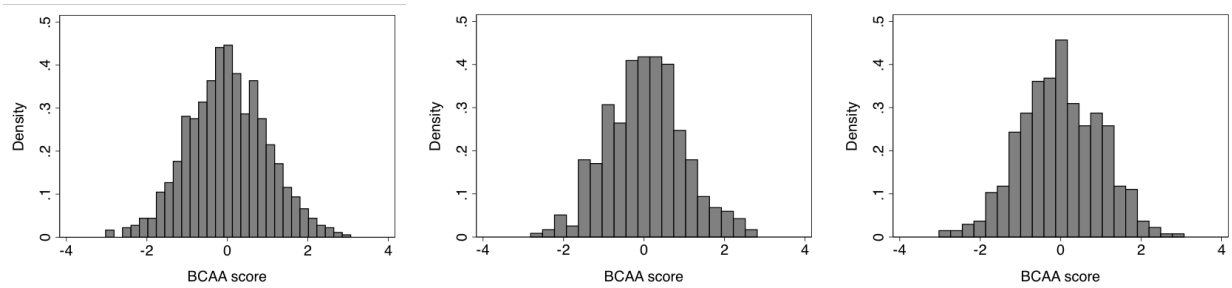


Supplemental Figure 4-2. Correlations between maternal plasma branched-chain amino acids (BCAAs)



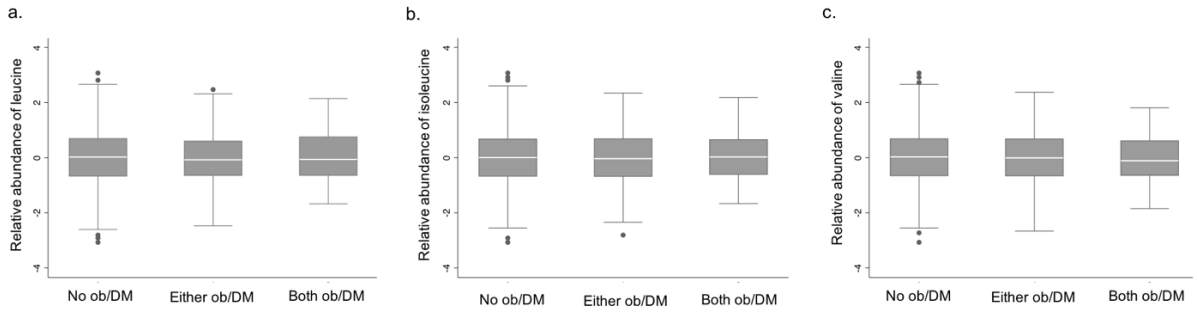
a. correlation between leucine and valine, b. correlation between leucine and isoleucine, c. correlation between valine and isoleucine

Supplemental Figure 4-3. Distribution of maternal plasma branched-chain amino acids (BCAA) score overall and by child's sex

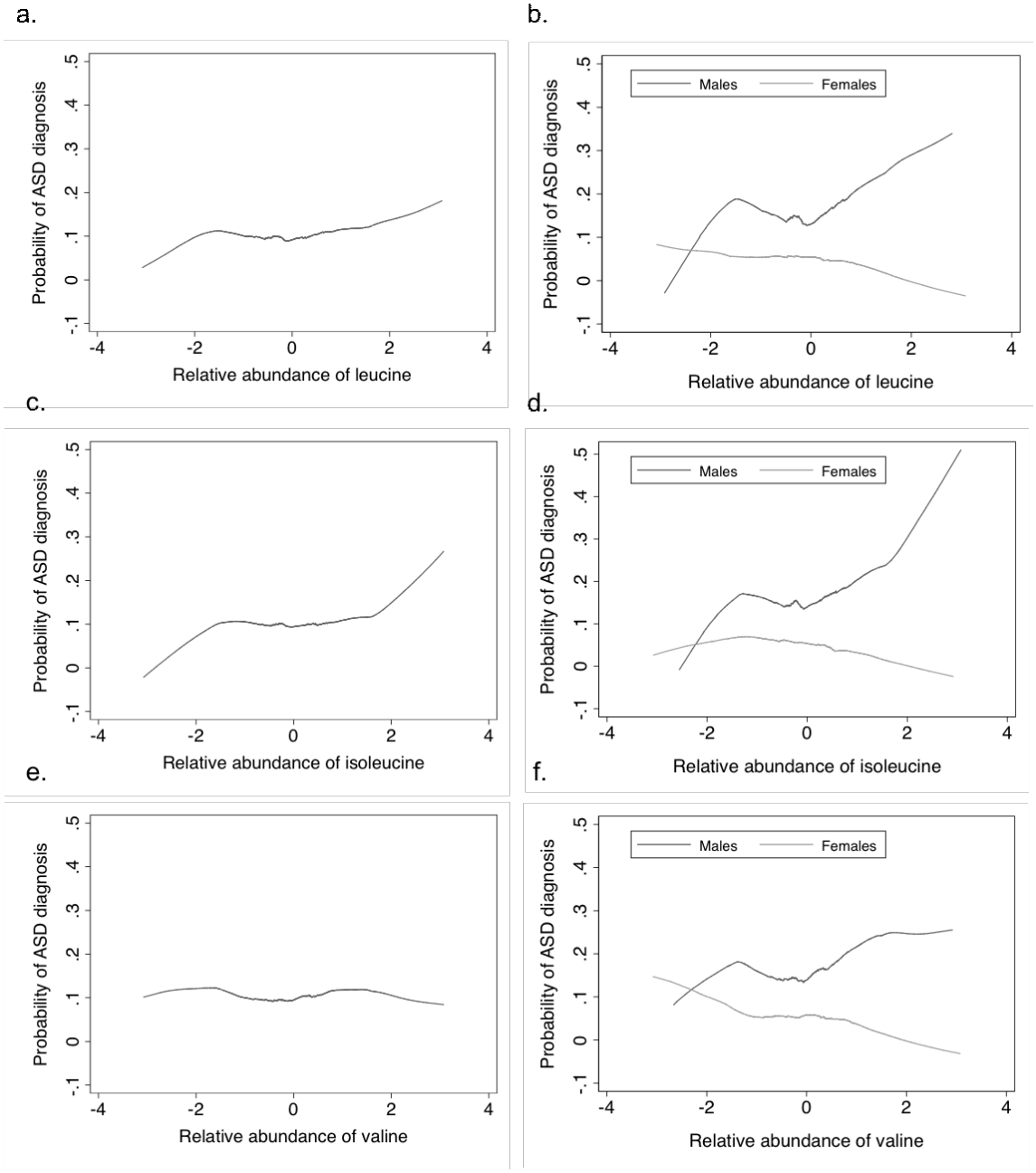


a. overall distribution of BCAA, b. distribution of BCAA among males, c. distribution of BCAA among females

Supplemental Figure 4-4. Maternal plasma branched-chain amino acids (BCAAs) stratified by maternal obesity/diabetes (ob/DM) status

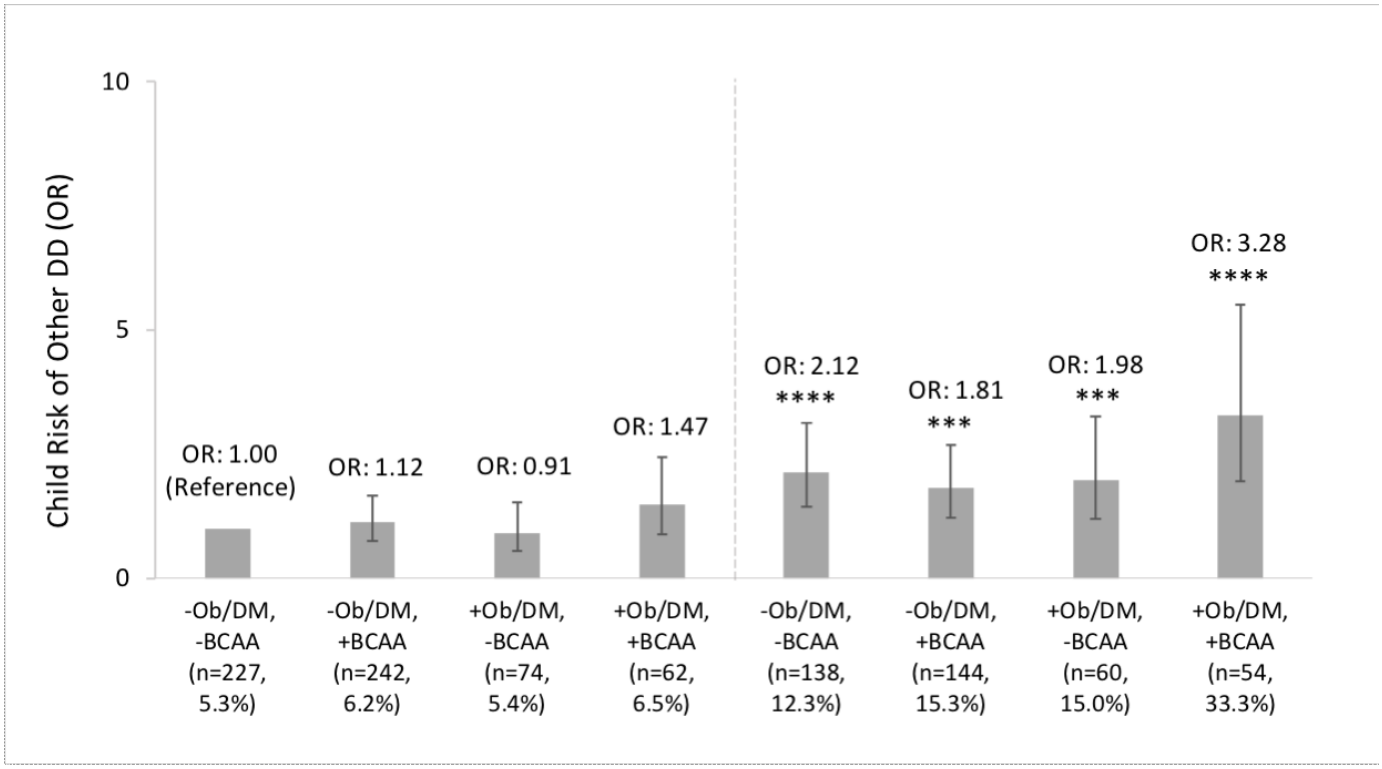


Supplemental Figure 4-5. Association of maternal plasma branched-chain amino acids (BCAAs) with risk of child ASD, overall and by child's sex



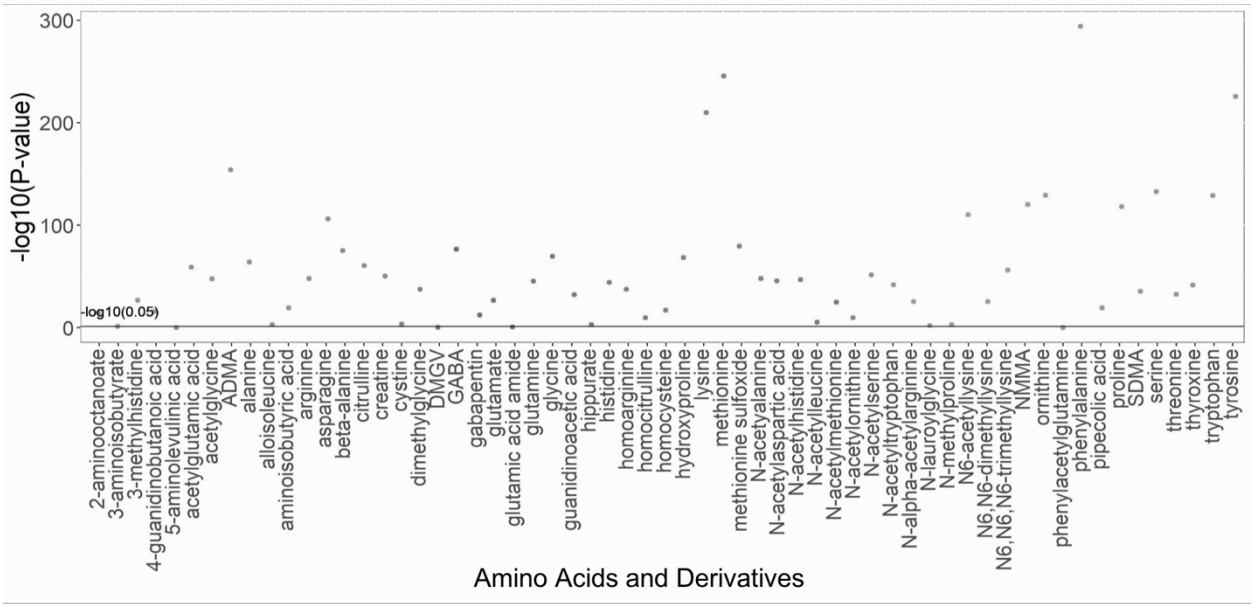
a. association between relative abundance in maternal plasma leucine and probability of ASD diagnosis in children, b. association between relative abundance in maternal plasma leucine and probability of ASD diagnosis by child's sex, c. association between relative abundance in maternal plasma isoleucine and probability of ASD diagnosis, d. association between relative abundance in maternal plasma isoleucine and probability of ASD diagnosis by child's sex, e. association between relative abundance in maternal plasma valine and probability of ASD diagnosis, f. association between relative abundance in maternal plasma valine and probability of ASD diagnosis by child's sex

Supplemental Figure 4-6. Joint association of maternal plasma branched-chain amino acid (BCAA) score, obesity/diabetes status, and child's sex on child other DD risk



-Ob/DM: No obesity/diabetes
+Ob/DM: Any obesity/diabetes
-BCAA: Below median branched-chain amino acid score
+BCAA: Above median branched-chain amino acid score
(n, % other DD); *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001

Supplemental Figure 4-7. Manhattan plot of associations between branched-chain amino acid (BCAA) score and other amino acids and derivatives



CHAPTER 5 MATERNAL DYSLIPIDEMIA, PLASMA BRANCHED-CHAIN AMINO ACIDS, AND THE RISK OF CHILD AUTISM SPECTRUM DISORDER: EVIDENCE OF SEX DIFFERENCE

ABSTRACT

Background

We and others have shown that maternal obesity and diabetes and elevated branched-chain amino acids (BCAAs) are risk factors of child Autism Spectrum Disorder (ASD). In contrast to the well-observed association of obesity and diabetes with ASD, the role of maternal dyslipidemia in ASD has not been well-studied. We aimed to examine the joint associations of maternal plasma cholesterols and BCAAs on child ASD risk and to determine whether these associations differed by child's.

Methods

We analyzed data from 829 mother-infant pairs, a subset of the Boston Birth Cohort with pertinent data. Maternal plasma cholesterols were measured using standard clinical methods and BCAAs were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in samples collected 24-72 hours postpartum. A composite BCAA score was created using factor analysis and dichotomized at the median, and specific cholesterol subtypes were dichotomized using clinical cut-points (HDL-C <50 mg/dl, TC ≥240 mg/dl, LDL-C ≥160, non-HDL-C ≥190 mg/dl). Logistic regression was used to explore the joint association of maternal cholesterols and

BCAA with child ASD among the overall sample and by sex, adjusting for important covariables, including maternal obesity and diabetes.

Results

Maternal plasma cholesterols by themselves were not associated with ASD. There was evidence of low maternal high-density lipoprotein cholesterol (HDL-C, <50 mg/dl) enhancing the effect of male sex on the risk of ASD, though tests for interaction were not significant. Among mothers with low HDL-C, high maternal BCAA score was associated with higher risk of child ASD (OR 4.67, 95% CI 1.33, 16.36). This association was consistent with significant interactions of all three BCAAs with HDL-C on the risk of child ASD. Compared to mothers with high HDL-C, low BCAAs, and a female child, mothers with low HDL-C, elevated BCAAs, and a male child were at the highest risk for child ASD (OR 4.78, 95% CI 2.12, 10.78) among all other combinations. Elevated risks associated with non-HDL and low-density lipoprotein cholesterols were not as robust.

Conclusions

In this US urban, low income prospective birth cohort, our findings suggest that low maternal HDL-C, high maternal plasma BCAAs, and male sex can jointly increase child risk of ASD. Additional studies are necessary to understand the mechanisms behind maternal HDL-C and BCAA involvement in ASD and their role in the profound sex disparity observed in ASD.

INTRODUCTION

Autism Spectrum Disorders (ASDs) present a wide array of symptoms characterized by impaired social interaction, deficits in communication, and restricted and stereotyped behaviors and interests.[1] ASD is a complex disorder and its etiology and underlying mechanisms remain unknown. While genetics is believed to play an important role, there are many environmental factors that have been associated with ASD.[2]

Cholesterols, within normal range, play important roles in brain function, mainly in regulating cell membrane permeability and the formation of synapses. Twenty-five percent of bodily cholesterol is found in the brain with 70% of brain cholesterol incorporated into myelin, the protective sheath for neuronal axons.[3] Maternal circulating cholesterols can be transported across the placenta and play important roles during fetal neurodevelopment.[4] For example, they are essential components of cellular membranes and also precursors for steroid hormones in the fetus. Disruptions in the level of certain cholesterols can result in adverse birth outcomes, including pre-term birth.[5] Abnormal maternal HDL-C and triglycerides measured post-delivery have been linked to attention deficit hyperactive disorder (ADHD) in children, the most common co-occurring condition with ASD.[6] However, there are no studies reporting on the association between maternal dyslipidemia and development of ASD in children.

We and others have previously shown that maternal obesity and diabetes contribute to an elevated risk of the child developing ASD, especially among male children.[7-9] Dyslipidemia is highly associated with obesity and diabetes and contributes to all definitions of the metabolic syndrome.[10] About a quarter of US women of reproductive age have higher than normal low-density lipoprotein cholesterol (LDL-C) levels (≥130 mg/dl) and 13% have lower than normal

high-density lipoprotein cholesterol (HDL-C) levels (<40 mg/dl).[11] The goal of this study was to examine the independent and joint effects of maternal dyslipidemia, branched-chain amino acids (BCAAs), and child's sex on child risk of ASD.

The BCAAs – leucine, isoleucine, and valine – are essential amino acids making up approximately a third of essential amino acids in muscle and are also involved in cellular signaling and modulation of glucose homeostasis and body weight.[12] Elevated levels have also been shown to predict type 2 diabetes mellitus[13] and are linked with increased risk of an atherogenic profile.[14] BCAAs are also associated with cholesterol metabolism.[15,16] Plasma cholesterol increased in patients with sepsis when given large dose of BCAAs, while other amino acids, fat, and glucose did not have an impact.[15] However, only a few studies exist on BCAA-ASD associations and with small sample sizes.[17-19] Further, there is a lack of inter-generational studies examining the joint association of maternal lipid profile and BCAA with child ASD.

We therefore examined whether maternal dyslipidemia is associated with the risk of ASD and sought to clarify whether maternal dyslipidemia can interact with BCAAs to affect child risk of ASD. As there is a sex disparity in the prevalence of ASD, we also investigated whether the associations differed by sex of the child.

METHODS

Participants and Data Collection

This study analyzed the existing data from the Boston Birth Cohort (BBC), an ongoing prospective cohort study at the Boston Medical Center (BMC) initiated in 1998. In the parent study, mother-infant pairs were recruited at birth using a rolling enrollment and followed up

from birth up to 21 years of age. The current study includes children followed up between 2004 – 2017. Detailed recruitment and follow-up procedures have been previously published.[8] The Johns Hopkins Bloomberg School of Public Health and the Boston University Medical Center Institutional Review Boards (IRB) approved the recruitment, follow-up studies, and protocols.

Briefly, at the time of enrolment, 24-72 hours post-partum, written informed consent was obtained from all participants. Mothers with multiple gestations or who became pregnant due to *in vitro* fertilization were excluded from the analysis as were babies with chromosomal abnormalities or major birth defects. Maternal blood was collected at the time of enrolment in a non-fasted state. Maternal and newborn prenatal and perinatal information were obtained via a maternal postpartum questionnaire interview and medical record review. Child postnatal information was obtained via child follow-up questionnaires completed by maternal interviews and child medical record review.

Of 3,163 mother-infant pairs in the BBC who were enrolled at birth and followed postnatally at the BMC, 829 (86 children with ASD and 743 typically developing (TD) children) who had complete data of the key variables were included in the analyses (Supplemental Figure 5-1). Electronic Medical Records (EMR) were used to define ASD case status as per primary and secondary diagnoses using ICD-9 or ICD-10 codes (Supplemental Table 5-1). Specifically, ASD was defined for children who were ever diagnosed with autism (299.00), Asperger syndrome (299.80), and/or pervasive developmental disorder - not otherwise specified (299.90). Though a systematic screening for ASD was not conducted, all children were evaluated by highly experienced staff at the BMC autism evaluation program who are in regular communication with primary care physicians at the medical center. Attention deficit hyperactivity disorder (ADHD), developmental delays, or intellectual disabilities without an ASD diagnosis were classified as

other developmental disorders. Children with no ASD or other developmental disorders were classified as typically developing (TD). Given the focus of this study, children with other developmental disabilities other than ASD were excluded from the analyses. Our study sample further excluded mothers missing key covariates and cholesterol measurements.

Serum total cholesterol (TC), HDL-C, and triglycerides were measured using standard clinical methods.[20] LDL-C was calculated using the Friedewald equation.[21] As the accuracy of TC, LDL-C, and TG are dependent on a fasted blood sample, this analysis focused mainly on maternal HDL-C. Quantitative profiling of maternal plasma metabolites, including the BCAAs, was conducted by the Harvard-MIT Broad Institute Metabolite Profiling Laboratory using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Statistical Analysis

Clinical and demographic characteristics for both mother and child were compared for the ASD and TD groups using the t-test and chi-squared test for continuous and categorical variables, respectively. For major covariates, missing values were combined into the largest group. Metabolite intensity levels were inverse-normally transformed for all subsequent analyses and metabolite values below the limit of detection were imputed with one-half the limit of detection. A BCAA score variable was created based on factor analysis of all three BCAAs using the Anderson-Rubin Method.[22] We explored the association between maternal dyslipidemia and child risk of ASD using logistic regression and further assessed for joint effects with sex and maternal plasma BCAAs, independently and altogether. Plasma HDL-C was evaluated in the main analysis because it is unaffected in a non-fasted state, unlike the other cholesterols. For joint effects and effect modification analyses, the reference category was the combination of high

HDL-C with female sex (or below median maternal BCAA levels) and was compared to three other groups: 2. low HDL-C with female sex (or below median BCAAs) 3. high HDL-C with male sex (or above median BCAAs with), and 4. low HDL-C with male sex (or above median BCAAs). The relative excess risk due to interaction (RERI) was used to show departure from the additive effects of BCAAs and HDL-C or sex.[23] Tests for multiplicative interaction were also conducted. All analyses were conducted using Stata v14.0 (Stata Corporation, College Station, TX, USA).

To define dyslipidemia, the cholesterol variables were dichotomized by their clinical cut-offs. The cut-off used for HDL-C was <50 mg/dl, for TC was ≥240 mg/dl, and for LDL-C was ≥160 mg/dl.[24,25] The non-HDL-C variable was created by subtracting HDL-C from TC and using a clinical cut-point of ≥190 mg/dl.[25]

Key maternal covariates in this analysis included: age at delivery (<20, 20-20, 30 and older), race-ethnicity (black, white, Hispanic, or other), smoking during pregnancy ("never smoked," "ever smoked," or "continuous smoking" within three months prior to conception), parity (nulliparous vs multiparous), education ("high school or less" vs "some college or more"), obesity/diabetes mellitus (ob/DM) (categorized into 1. no obesity (BMI ≥30 kg/m^2), no type 2 diabetes mellitus or gestational diabetes (DM, collectively); 2. either obesity or DM; 3. both obesity and DM). Key child covariates included: sex (female vs male), and gestational age and birthweight (categorized into 1. full term (≥37 weeks of gestation) and non-low birth weight (non-LBW; ≥2500g); 2. full term and LBW; 3. preterm and non-LBW; 4. preterm and LBW).

RESULTS

In total, 829 mother-infant pairs were included in this study consisting of 86 children with ASD

and 743 TD children. Table 5-1 presents maternal and child characteristics by child ASD status. Mothers with ASD children were approximately two years older (p=0.01) and more obese (p=0.01) than mothers with TD children. They were also more likely to have diabetes, though this difference was marginally significant (p=0.05). There was a 3:1 male to female ratio in the prevalence of ASD (p<0.0001) and ASD children had shorter gestation (p<0.0001) and lower birth weight (p=0.04) than their TD counterparts. When considered by themselves, maternal cholesterol levels, BCAA levels, smoking, race/ethnicity, parity, or education were not associated with child ASD risk. To evaluate potential selection bias of the study sample, Supplemental Table 5-2 compares maternal and child characteristics for included and excluded participants; there were significantly more black mothers included in the study than other race (p<0.0001) and fewer mothers who had ever smoked or continued to smoke (p=0.001). A significantly greater number of infants included in the study were female (p<0.01), born full term (≥37 weeks) (p<0.0001), and at a normal weight (>2500 g) (p<0.0001). There were no significant in maternal and child characteristics by level of maternal HDL-C (Supplemental Table 5-3) and maternal cholesterols did not significantly vary by ASD case status (Supplemental Figure 5-2).

Maternal cholesterols had joint effects with child's sex on child risk of ASD, even after adjustment for pertinent covariables, including maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight (Table 5-2). The effect of HDL-C, non-HDL-C, and LDL-C on child risk of ASD all differed by child's. Males whose mothers had low HDL-C were at highest risk of ASD (odds ratio (OR): 4.20; 95% CI 2.02, 8.73), followed by males whose mothers had high HDL-C (OR: 3.70; 95% CI 2.05, 6.67), compared to females whose mothers had high HDL-C. Further, the risk of child ASD was higher for males

than females more so among mothers with low HDL-C than with high HDL-C (crude OR: 5.57; 95% CI 1.81, 17.11 vs crude OR: 3.53; 95% CI 1.99, 6.23, respectively). A similar pattern was found for elevated LDL-C and non-HDL-C for crude stratified ORs by child's. However, assessment of maternal cholesterol-infant sex interactions on additive and multiplicative scales were not significant for any of these cholesterol measures.

Maternal HDL-C also modified the effect of maternal plasma BCAAs on child risk of ASD (Table 5-3). Among mothers with low levels of HDL-C, the crude OR for the effect of high BCAA score on ASD risk was over four-fold (OR 4.67; 95% CI: 1.33, 16.36). For isoleucine, this effect was over six-fold and for valine over seven-fold. Assessment of BCAA-HDL-C interactions were significant on the additive scale (BCAA score RERI 0.12; 95% CI: 0.03, 0.20) and multiplicative scale (BCAA score OR: 6.13; 95% CI: 1.42, 26.42) across all three BCAAs. Sensitivity analyses showed consistent results (Supplemental Tables 5-4 – 5-5). Analyses on TC, LDL-C, and non-HDL-C are also presented (Supplemental Tables 5-6 – 5-14), manifesting similar trends, but not as robust as HDL-C.

Finally, we examined the joint effect of HDL-C, BCAA, and child's sex on child ASD risk. Figure 5-1 illustrates the association of maternal HDL-C levels with child risk of ASD, stratified by maternal plasma BCAA status as defined by the BCAA score, among the overall sample, and among males and females, respectively. Most notably, in mothers with high BCAA scores, the risk of ASD was higher with lower HDL-C concentrations, decreasing steadily as HDL-C increased. This pattern was more exaggerated among male children. Figure 5-2 displays similar patterns for the other cholesterol measures. The group with all three exposures combined – males whose mothers had a high BCAA score and low HDL-C – had the greatest risk of ASD,

compared to the reference group (crude OR: 4.78; 95% CI 2.12, 10.78) (Supplemental Figure 5-3).

DISCUSSION

Main Findings

To our knowledge, this is the first study of this kind in US urban, low income prospective birth cohort. We found that low maternal HDL-C, high maternal plasma BCAA concentrations, and male sex can additively or multiplicatively increase child risk of ASD. This study extends previous studies by us and others on the role of maternal metabolic factors in child ASD.

Interpretation

Though the role of maternal obesity and diabetes in child ASD has received increasing attention due to accumulating evidence, there is a lack of studies linking maternal cholesterols to ASD. However, as reviewed below, it is biologically plausible that abnormal maternal cholesterols may affect the risk of child ASD. While cholesterols are be formed *de novo* by the fetus, maternal cholesterols are also transported via placenta, at least during the first trimester, and enter fetal circulation.[4] A recent meta analyses of maternal dyslipidemia reported that both low and high levels of cholesterol were associated with preterm birth,[5] which is also highly associated with ASD.[26,27] Low maternal cholesterol has also been linked with low birthweight and microcephaly.[28] In cases of maternal hypercholesterolemia, fetal cholesterols also become elevated, and this increased exposure has been shown to predispose the offspring to atherosclerosis later in life.[29] Elevated maternal cholesterol has been consistently linked with adverse adult offspring outcomes, including atherosclerosis and cardiovascular disease,[30] which

are co-occurring conditions in individuals with ASD.[31] It is possible that selective diets and food sensitivities in ASD could partially account for the pathophysiology of these inflammatory conditions.[32]

Adequate levels of cholesterol are necessary for proper membrane myelination during fetal development. Reduced levels of myelin have been seen in individuals with Fragile X syndrome, a disorder closely related to ASD.[33] However, there is inconsistent evidence for altered cholesterol levels in ASD individuals; some studies have found higher total and LDL-C levels among ASD individuals while others report normal or lower cholesterol compared to controls.[34,35] This may be due to the differing subtypes and etiologies of ASD.

Genetic defects of cholesterol biosynthesis have severe impacts on development and survival of the fetus.[35] Smith–Lemli–Opitz Syndrome (SLOS) is a milder genetic disorder characterized by a defect in cholesterol synthesis and is associated with autistic traits. One study reported around 20% of children with ASD had hypocholesterolemia.[36] Abnormal cholesterol metabolism in SLOS and ASD may lead to dysfunction in serotonin signaling, as cholesterol modulates the activity of serotonin, especially in its transport. Serotonin has been implicated in altered social behaviors characteristic of SLOS and ASD. A mouse model for SLOS supports this finding.[37] A recent study also found that adult rats prenatally exposed to valproic acid to induce autistic-like traits had sex-specific differences in brain cholesterol metabolism compared to unexposed rats.[38]

With the exception of vitamin D, all steroid hormones are formed from cholesterol precursors. Steroids have been implicated in the etiology of anxiety and mood disorders as well as ASD.[36] There is growing literature on maternal vitamin D deficiency during pregnancy and ASD.[39,40] Serotonin, oxytocin, and vasopressin all contain a vitamin D response element (VDRE).[40] The

genes encoding production of each require vitamin D to activate them. All three of these hormones are also modulated by cholesterol. Although cholesterol is not a precursor for vitamin D, they are both derived from the same molecule, 7-dehydrocholesterol (7DHC).[33] The 7DHC reductase gene (DHCR7) is the same as that implicated in SLOS. Gillberg et al. (2017) hypothesized there may be an association between ASD and this "branching point," possibly explaining the links between ASD and cholesterol and ASD and vitamin D.[33] Pregnant women with inadequate levels of 25-hydroxyvitamin D during the first trimester had significantly increased TC, LDL-C, and TC/HDL-C ratio compared to pregnant women with sufficient levels of the vitamin.[41]

It is a well-observed phenomenon that there is a profound sex disparity in ASD, with male to female ratio of 3:1.[42] However, the underlying reasons and mechanisms for the sex difference are poorly understood. In the present study, for the first time, we showed that the effect of low maternal HDL-C on child ASD risk was most pronounced in male children. This link between cholesterol and sex is supported by a study that reported estrogen is able to compensate for insufficient maternal vitamin D, potentially rescuing female fetuses from developing psychiatric disorders, including ASD.[40] Thus, our finding puts forth new indications for future research.

This study demonstrated that maternal cholesterols by themselves were not associated with child risk of ASD. However, HDL-C, non-HDL-C, and LDL-C did modify maternal effect of BCAAs on child ASD risk with significant additive and multiplicative interactions.

BCAAs are highly correlated with obesity and T2DM and predictive of T2DM.[13,43] Yet, there is limited evidence linking cholesterol and BCAAs. One study showed plasma cholesterol levels increased in septic patients given a large dose of BCAAs, while other amino acids, fat, and

glucose were not impacted.[15] This suggests BCAA metabolites contribute to cholesterol synthesis in an inflammatory state. Both BCAA and lipid metabolism involve the tricarboxylic acid (TCA) cycle in the mitochondria and are thus dependent on the proper functioning of each other. Disturbances in lipid metabolism have been shown to yield elevated serum concentrations of BCAAs.[16] These disturbances in energy metabolism have the potential to cause oxidative stress and inflammation. BCAAs, especially leucine, are inducers of mammalian target of rapamycin (mTOR), an important kinase involved in cell growth, protein synthesis, and energy balance.[44] Thus, together with low HDL-C levels and child male sex, BCAAs have the potential to increase the risk of child ASD. Our results showed that male children whose mothers had low HDL-C levels and low BCAA concentrations, were not at significant risk compared to the reference group (female children whose mothers had high HDL-C levels and low BCAA concentrations) (Supplemental Figure 5-3). However, male children whose mothers had low HDL-C levels and *high* BCAA concentrations had a significantly increased risk, suggesting elevated BCAA concentrations are key factors in increasing the risk under these conditions. This analysis was unadjusted for key covariates and conducted as exploratory research.

Strengths and Limitations

This is a relatively large, prospective, longitudinal, and inter-generational study design coupled with maternal biomarkers of lipids and BCAAs. It is the first to examine the joint effects of maternal cholesterol and metabolites on the risk of her child developing ASD. This study also brought to light potential biomarkers associated with sex differences observed in ASD. Limitations of this study include a one-time post-delivery measurement of maternal BCAAs and cholesterols in a non-fasted state. Apart from HDL-C, cholesterol measurements in a non-fasted state are not as reliable, especially triglycerides, which we did not analyze. Though our sample

size was among the largest for a prospective study of its kind, we were not able to examine stratified odds ratios adjusted for pertinent covariables. We caution the number of cases is very small in certain category and the observed associations may not be reliable. As such, our findings should be regarded as hypothesis-generating and warrant further investigation and confirmation.

Implications for future research

While most current research on BCAAs in ASD has shown reduced levels in ASD cases versus controls, we are not aware of any that have examined the prospective effect of maternal BCAAs on child risk of ASD. The developmental origins of health and disease model asserts that in a womb environment of plenty, the fetus adapts to expect a similar postnatal environment.[45] Thus, fetuses exposed to excess levels of BCAAs in the womb may express fewer circulating concentrations postnatally. Other possible explanations of lower concentrations of BCAAs among individuals with ASD include food sensitivities and poor diet quality leading to insufficient intake of certain nutrients,[32] and possible mutations in the branched chain ketoacid dehydrogenase kinase (BCKDK), a key enzyme in BCAA catabolism.[46] Future studies including both maternal plasma measurements during specific trimesters of pregnancy as well as cord blood and postnatal child plasma cholesterols and BCAAs may help clarify the inter-generational link of cholesterols and BCAAs with child ASD risk.

Conclusions

The results of this longitudinal, prospective birth cohort study showed that while maternal plasma cholesterols were not associated with the risk of child ASD, they had a joint effect with child's sex on this risk. Low HDL-C and elevated maternal plasma BCAAs jointly increased the risk as well and together with child male sex, the three factors together had the greatest effect on

the risk of child development of ASD. Future studies with larger sample sizes and additional time points for HDL-C and BCAAs throughout pregnancy, from fetal cord blood, and during early childhood may further elucidate the role of maternal and fetal/child cholesterols and BCAAs in the risk of child ASD.

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Table 5-1. Maternal and child characteristics by child autism spectrum disorder (ASD) status (Typically Developing (TD) vs. ASD) in the Boston Birth Cohort

Maternal Characteristics	Total (N=829)	TD (N=743)	ASD (N=86)	P-value[a]
Age (years), mean (SD)[b]	28.29 (6.51)	28.09 (6.51)	30.00 (6.34)	0.010
≤25	298 (35.95)	274 (36.88)	24 (27.91)	0.257
26-35	390 (47.04)	344 (46.30)	46 (53.49)	
≥36	141 (17.01)	125 (16.82)	16 (18.60)	
Nulliparous, n (%)	362 (43.67)	327 (44.01)	35 (40.70)	0.344
Race or ethnicity, n (%)[c]				0.089
Black	585 (70.57)	533 (71.74)	52 (60.47)	
White	33 (3.98)	28 (3.77)	5 (5.81)	
Hispanic	156 (18.82)	132 (17.77)	24 (27.91)	
Other	55 (6.63)	50 (6.73)	5 (5.81)	
Education, n (%)				0.991
Below college degree	707 (85.28)	635 (85.46)	72 (83.72)	
College degree or above	116 (13.99)	104 (14.00)	12 (13.95)	
Missing	6 (0.72)	4 (0.54)	2 (2.33)	
Pre-pregnancy BMI, n (%)				
Mean (SD)	26.57 (6.59)	26.41 (6.42)	27.96 (7.91)	0.046
Normal weight (<25 kg/m^2)	388 (46.80)	354 (47.64)	34 (39.53)	0.019
Overweight (25 - <30 kg/m^2)	210 (25.33)	193 (25.98)	17 (19.77)	
Obese (≥30 kg/m^2)	194 (23.40)	165 (22.21)	29 (33.72)	
Missing	37 (4.46)	31 (4.17)	6 (6.98)	
Diabetes, n (%)[d]				0.053
No diabetes	736 (88.78)	665 (89.50)	71 (82.56)	
Diabetes	93 (10.76)	78 (10.50)	15 (17.44)	
Smoking, n (%)[e]				0.665
Never	702 (84.68)	632 (85.06)	70 (81.40)	
Quit	54 (6.51)	47 (6.33)	7 (8.14)	
Continuous	64 (7.72)	56 (7.54)	8 (9.30)	
Missing	9 (1.09)	8 (1.08)	1 (1.16)	
Total cholesterol, mean (SD)	218.38 (59.45)	218.54 (59.90)	216.97 (55.74)	0.818
High cholesterol (≥240 mg/dl), n (%)	260 (31.36)	235 (31.63)	25 (29.07)	0.628
LDL cholesterol, mean (SD)	125.72 (40.41)	125.74 (40.74)	125.55 (37.75)	0.968
High LDL (≥160 mg/dl), n (%)	146 (17.61)	134 (18.03)	12 (13.95)	0.347
HDL cholesterol, mean (SD)	62.77 (17.98)	63.08 (18.26)	60.02 (15.16)	0.135
Low HDL (<50 mg/dl), n (%)	201 (24.25)	179 (24.09)	22 (25.58)	0.760
Leucine (above median), n (%)	416 (50.18)	368 (49.53)	48 (55.81)	0.270
Isoleucine (above median), n (%)	417 (50.30)	372 (50.07)	45 (52.33)	0.692
Valine (above median), n (%)	416 (50.18)	366 (49.26)	50 (58.14)	0.119
BCAA score (above median), n (%)	410 (49.46)	362 (48.72)	48 (55.81)	0.213
Child Characteristics	**Total (N=829)**	**TD (N=743)**	**ASD (N=86)**	**P-value[a]**
Sex, n (%)				<0.0001
Male	381 (45.96)	317 (42.66)	64 (74.42)	
Female	448 (54.04)	426 (57.34)	22 (25.58)	
Gestational age, n (%)				<0.0001
Term (≥37 weeks)	709 (85.52)	645 (86.81)	64 (74.42)	
Late preterm (34-36 weeks)	64 (7.72)	60 (8.08)	4 (4.65)	

Early preterm (<34 weeks)	56 (6.76)	38 (5.11)	18 (20.93)	
Birth weight, n (%)				0.036
≥2,500 grams	676 (81.54)	613 (82.50)	63 (73.26)	
<2,500 grams	153 (18.46)	130 (17.50)	23 (26.74)	

SD, standard deviation; LDL, low-density lipoprotein; HDL, high-density lipoprotein

[a]P-values were obtained from χ^2 tests or t-tests; missing values for categorical variables were incorporated into the largest group

[b]Maternal age at time of delivery

[c]Black includes self-reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[d]Type 2 diabetes mellitus and/or gestational diabetes mellitus

[e]Never smokers were defined as mothers with no history of smoking three months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of three months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting three months prior to and throughout pregnancy

Table 5-2. Joint association of maternal plasma cholesterols and child's sex on the risk of child autism spectrum disorder (ASD)

	HDL-C (≥50mg/dl)		HDL-C (<50mg/dl)		
Child's sex	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) HDL-C within strata of Sex[a]
Female	18/345	1.00 (ref)	4/103	0.67 (0.22-2.09)	0.73 (0.24-2.22)
Male	46/283	**3.70 (2.05-6.67)**	18/98	**4.20 (2.02-8.73)**	1.16 (0.64-2.11)
OR (95% CI) Sex within strata of HDL-C[a]		**3.53 (1.99-6.23)**		**5.57 (1.81-17.11)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.03 (-0.06-0.13); P = 0.499 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.69 (0.46-6.19); P = 0.431					
	Non-HDL-C (<190 mg/dl)		Non-HDL-C (≥190 mg/dl)		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) Non-HDL-C within strata of Sex[a]
Female	19/358	1.00 (ref)	3/90	0.64 (0.18-2.26)	0.62 (0.18-2.13)
Male	54/307	**4.14 (2.34-7.33)**	10/74	**2.77 (1.17-6.52)**	0.73 (0.35-1.52)
OR (95% CI) Sex within strata of Non-HDL-C[a]		**3.81 (2.20-6.58)**		**4.53 (1.20-17.13)**	
Measure of interaction on additive scale: RERI (95% CI) = -0.03 (-0.13-0.07); P = 0.536 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.04 (0.24-4.53); P = 0.958					
	LDL-C (<160 mg/dl)		LDL-C (≥160 mg/dl)		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) LDL-C within strata of Sex[a]
Female	19/368	1.00 (ref)	3/80	0.77 (0.22-2.71)	0.72 (0.21, 2.48)
Male	55/315	**4.23 (2.40-7.46)**	9/66	**2.94 (1.21-7.11)**	0.75 (0.35, 1.60)
OR (95% CI) Sex within strata of LDL-C[a]		**3.89 (2.25-6.71)**		**4.05 (1.05-15.64)**	
Measure of interaction on additive scale: RERI (95% CI) = -0.03 (-0.13-0.07); P = 0.536 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.91 (0.20-4.01); P = 0.896					

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol
ORs adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight unless otherwise noted
[a]Stratified ORs not adjusted

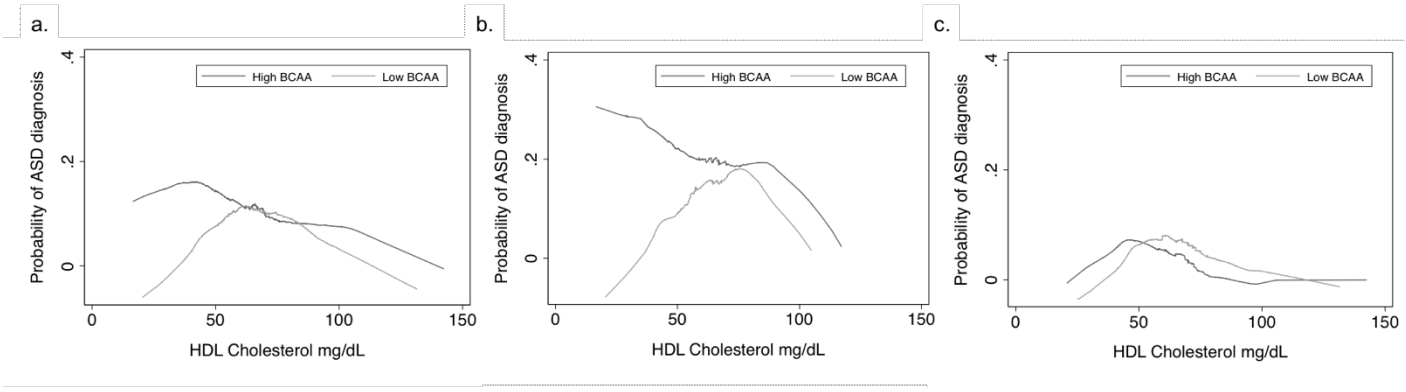
Table 5-3. Joint association of maternal plasma branched-chain amino acids (BCAAs) and high-density lipoprotein cholesterol (HDL-C) on the risk of child autism spectrum disorder (ASD)

	Leucine below median		**Leucine above median**		
Maternal HDL-C	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL-C[a]
High HDL-C	35/336	1.00 (ref)	29/292	0.98 (0.56-1.70)	0.95 (0.56-1.59)
Low HDL-C	3/77	**0.27 (0.07-0.97)**	19/124	1.56 (0.81-2.99)	**4.46 (1.27-15.63)**
OR (95% CI) HDL-C within strata of BCAA[a]		0.35 (0.10-1.16)		1.64 (0.88-3.05)	
Measure of interaction on additive scale: RERI (95% CI) = **0.12 (0.03-0.20); P = 0.007** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **5.95 (1.38-25.72); P = 0.017**					
	Isoleucine below median		**Isoleucine above median**		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL-C[a]
High HDL-C	39/338	1.00 (ref)	25/290	0.73 (0.41-1.27)	0.72 (0.43-1.23)
Low HDL-C	2/74	**0.16 (0.03-0.74)**	20/127	1.40 (0.74-2.64)	**6.73 (1.53-29.68)**
OR (95% CI) HDL-C within strata of BCAA[a]		**0.21 (0.05-0.90)**		**1.98 (1.06-3.72)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.15 (0.07-0.23); P = <0.001** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **12.21 (2.23-66.77); P = 0.004**					
	Valine below median		**Valine above median**		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL-C[a]
High HDL-C	34/336	1.00 (ref)	30/292	1.06 (0.61-1.85)	1.02 (0.61-1.71)
Low HDL-C	2/77	**0.17 (0.04-0.80)**	20/124	1.73 (0.91-3.30)	**7.21 (1.64-31.79)**
OR (95% CI) HDL-C within strata of BCAA[a]		0.24 (0.06-1.01)		1.70 (0.91-3.09)	
Measure of interaction on additive scale: RERI (95% CI) = **0.13 (0.05-0.21); P = 0.002** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **9.50 (1.78-50.88); P = 0.009**					
	BCAA score below median		**BCAA score above median**		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL-C[a]
High HDL-C	35/340	1.00 (ref)	29/288	1.00 (0.57-1.74)	0.98 (0.58-1.64)
Low HDL-C	3/79	**0.26 (0.07-0.96)**	19/122	1.61 (0.84-3.10)	**4.67 (1.33-16.36)**

OR (95% CI) HDL-C within strata of BCAA[a]	0.34 (0.10-1.15)	1.65 (0.88-3.09)
Measure of interaction on additive scale: RERI (95% CI) = **0.12 (0.03-0.20); P = 0.006**		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **6.13 (1.42-26.42); P = 0.015**		

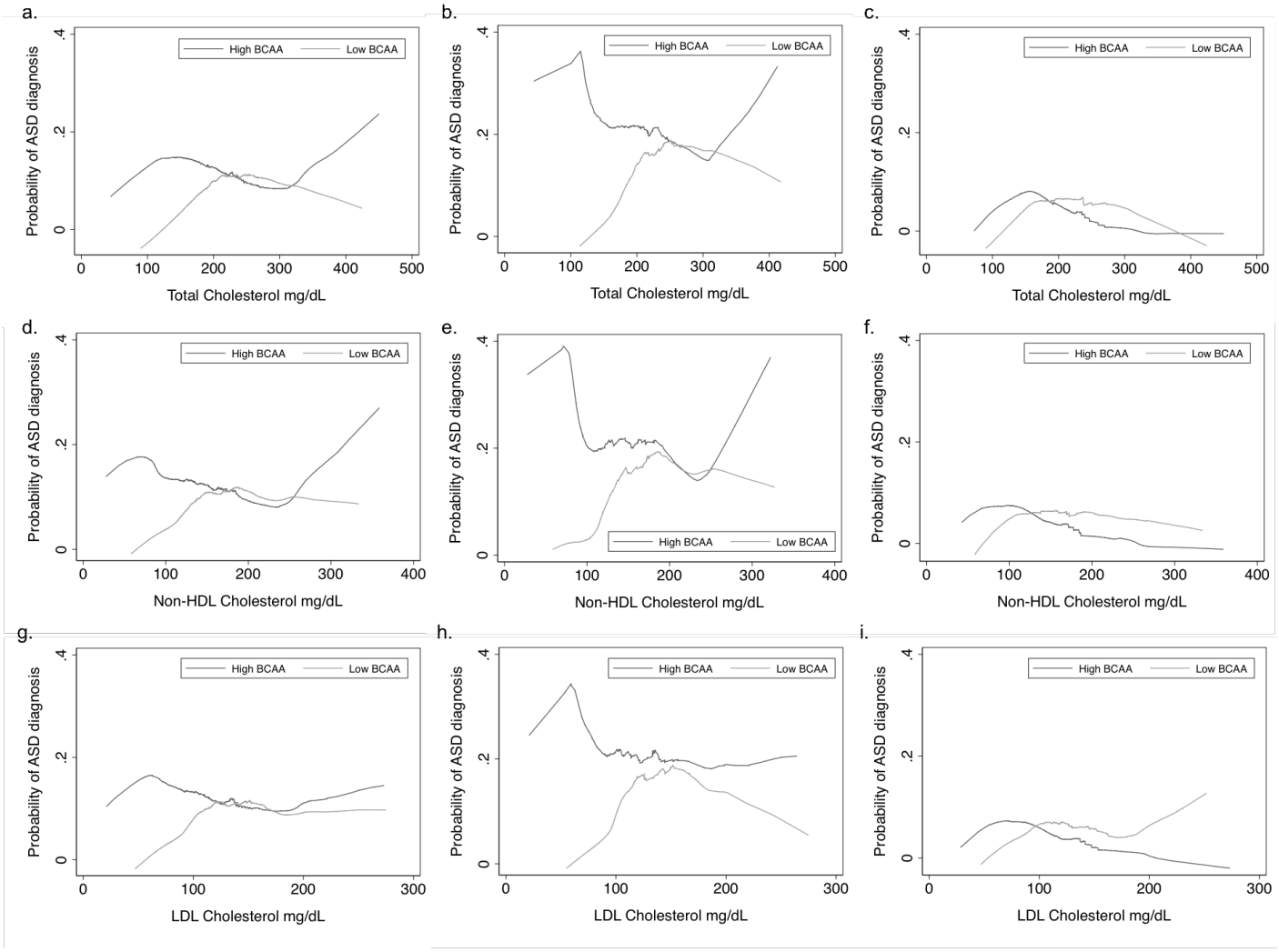
HDL-C, high-density lipoprotein cholesterol
Note: Low HDL-C <50 mg/dl
ORs adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight unless otherwise noted
[a]Stratified ORs unadjusted

Figure 5-1. Association of maternal HDL levels with risk of child autism spectrum disorder (ASD), stratified by maternal plasma branched-chain amino acid (BCAA) status defined by BCAA score, among overall sample, and among males and females, respectively



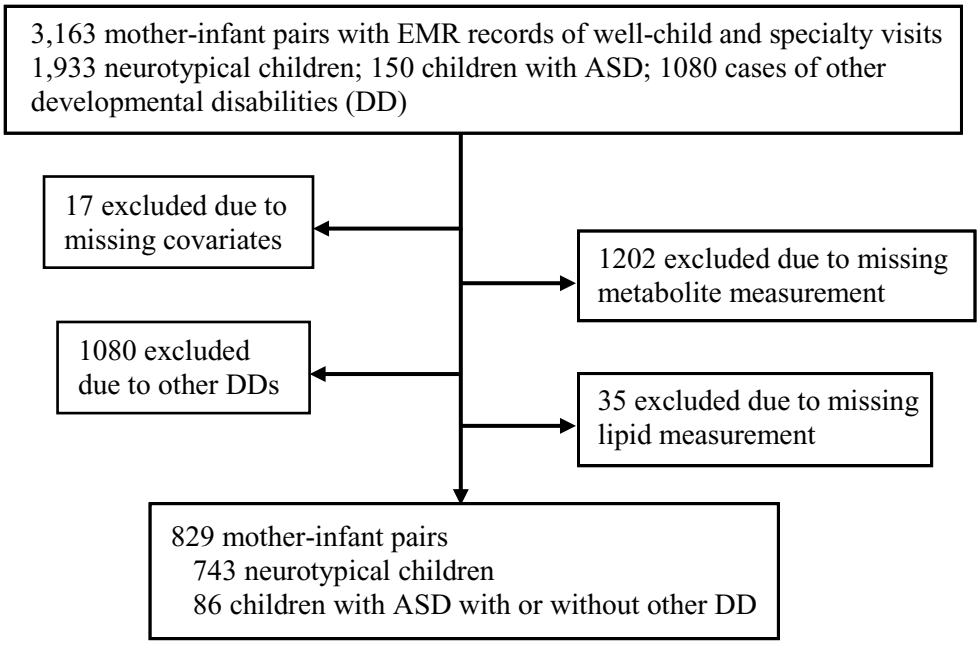
a. overall, b. male, c. female

Figure 5-2. Association of maternal total cholesterol, non-HDL cholesterol, and LDL levels with risk of child autism spectrum disorder (ASD), stratified by maternal plasma branched-chain amino acid (BCAA) status defined by BCAA score, among overall sample, and among males and females, respectively

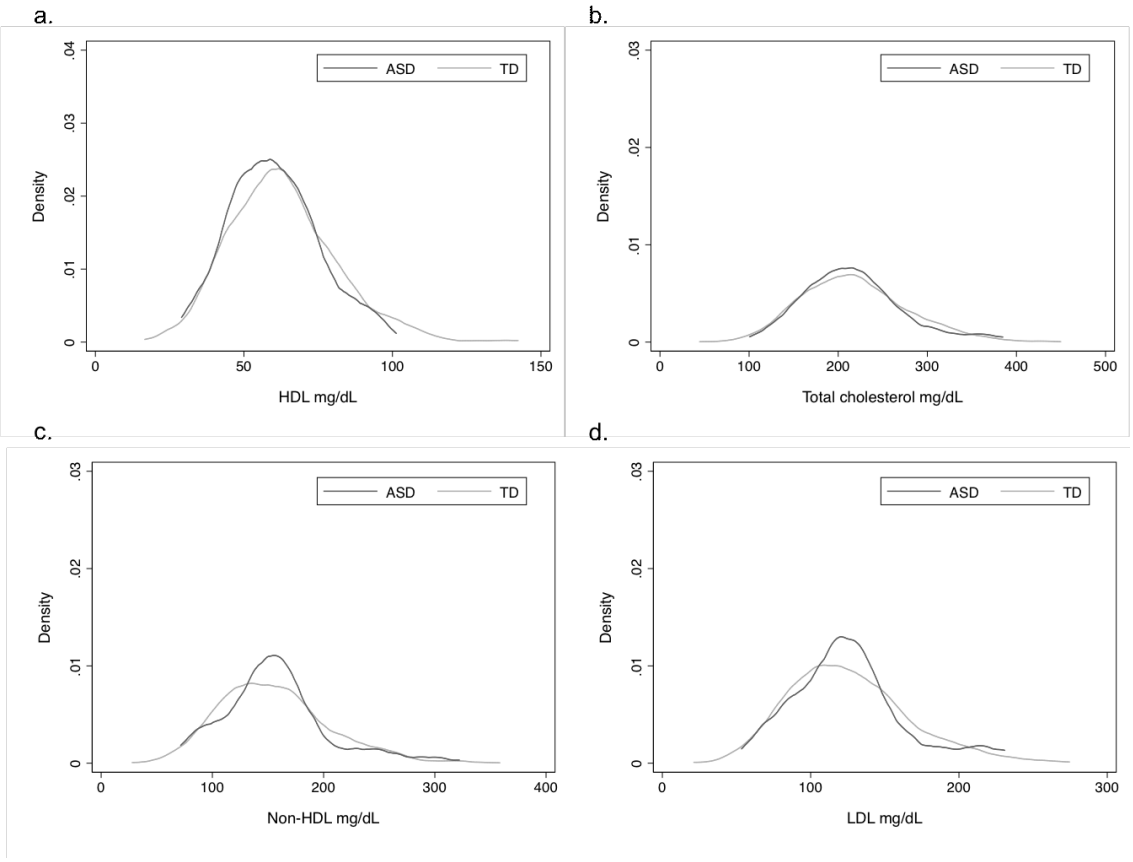


a. association of maternal TC with risk of child ASD by maternal plasma BCAA score in the overall sample, b. association of maternal TC with risk of ASD in male children by maternal plasma BCAA score, c. association of maternal TC with risk of ASD in female children by maternal plasma BCAA score, d. association of maternal non-HDL cholesterol with child risk of ASD by maternal plasma BCAA score in the overall sample, e. association of maternal non-HDL cholesterol with risk of ASD in male children by maternal plasma BCAA score, f. association of maternal non-HDL cholesterol with risk of ASD in female children by maternal plasma BCAA score, g. association of maternal LDL cholesterol with child risk of ASD by maternal plasma BCAA score in the overall sample, h. association of maternal LDL cholesterol with risk of ASD in male children by maternal plasma BCAA score, i. association of maternal LDL cholesterol with risk of ASD in female children by maternal plasma BCAA score

Supplemental Figure 5-1. Flowchart of study sample included and excluded in the analyses

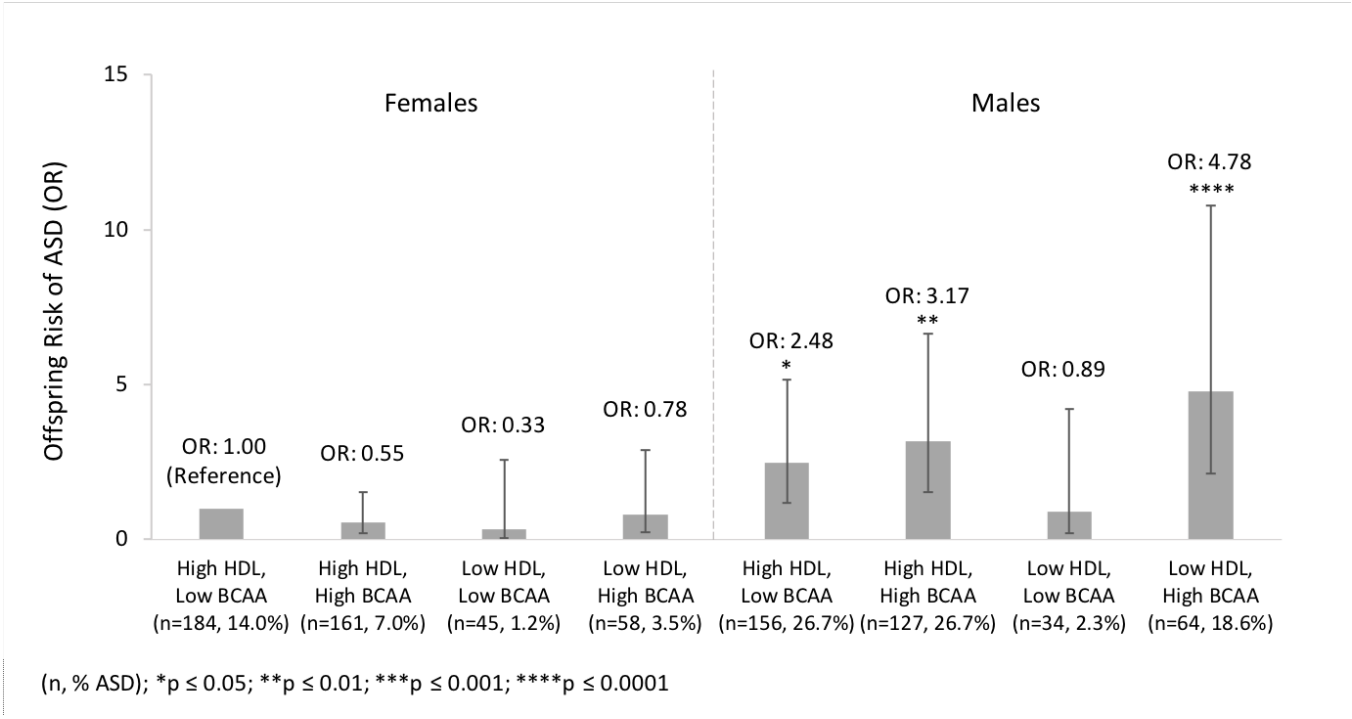


Supplemental Figure 5-2. Maternal cholesterols by child case status (autism spectrum disorder (ASD) and typically developing (TD) children)



a. Maternal high-density lipoprotein cholesterol (HDL-C) by ASD status b. Maternal total cholesterol by ASD status c. Maternal non- high-density lipoprotein cholesterol (non-HDL-C) by ASD status d. Maternal low-density lipoprotein cholesterol (LDL-C) by ASD status
Note: T-tests for all cholesterols were not significant

Supplemental Figure 5-3. Joint association of maternal plasma branched-chain amino acid (BCAA) score, maternal high-density lipoprotein cholesterol (HDL-C) status and child's sex on risk of child autism spectrum disorder (ASD)



(n, % ASD); *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001

Supplemental Table 5-1. List of ICD-9 and ICD-10 codes for the diagnosis of each developmental disorder

Developmental disorder	ICD-9 codes	ICD-10 codes
ASD	299.0, 299.00, 299.01, 299.8, 299.80, 299.81, 299.9, 299.90, 299.91	F84.0, F84.8, F84.9
ADHD	314.0, 314.00, 314.01, 314.1, 314.2, 314.8, 314.9	F90, F90.0, F90.1, F90.2, F90.8, F90.9
Developmental delays	315.0-315.9	F81.0, R48.0, F81.81, F81.2, F81.89, F80.1, F80.2, H93.25, F80.4, F80.81, F80.0, F80.82, F80.89, F82, F88, F81.9, F89
Intellectual disabilities	317-317	F70, F71, F72, F73, F78, F79

Supplemental Table 5-2. Maternal and child characteristics of Boston Birth Cohort participants excluded and included in the analysis

Characteristics	Total, N (%)	Excluded, N (%)	Included, N (%)	P-value[a]
Total	3138 (100.00)	2309 (73.58)	829 (26.42)	
Maternal age (years), mean (SD)[b]	28.64 (6.50)	28.60 (6.48)	28.29 (6.51)	0.234
Nulliparous, n (%)	1337 (42.61)	975 (42.23)	362 (43.67)	0.472
Race or ethnicity, n (%)[c]				<0.0001
Black	1998 (63.67)	1413 (61.20)	585 (70.57)	
White	227 (7.23)	194 (8.40)	33 (3.98)	
Hispanic	701 (22.34)	545 (23.60)	156 (18.82)	
Other	212 (6.76)	156 (6.80)	55 (6.63)	
Maternal education, n (%)				0.683
Below college degree	2690 (85.72)	1983 (85.88)	707 (85.28)	
College degree or above	426 (13.58)	310 (13.43)	116 (13.99)	
Missing	22 (0.70)	16 (0.69)	6 (0.72)	
Maternal BMI, n (%)				
Mean (SD)	26.58 (6.65)	26.59 (6.67)	26.57 (6.59)	0.950
Normal weight (<25 kg/m^2)	1452 (46.27)	1064 (46.08)	388 (46.80)	0.898
Overweight (25 - <30 kg/m^2)	813 (25.90)	603 (26.12)	210 (25.33)	
Obese (≥30 kg/m^2)	703 (22.40)	509 (22.04)	194 (23.40)	
Missing	170 (5.42)	133 (5.76)	37 (4.46)	
Maternal Diabetes, n (%)[d]				0.255
No diabetes	2751 (87.67)	2015 (87.27)	736 (88.78)	
Diabetes	387 (12.33)	294 (12.73)	93 (11.22)	
Maternal smoking, n (%)[e]				
Never	2542 (81.01)	1840 (79.69)	702 (84.68)	0.001
Quit	241 (7.68)	187 (8.10)	54 (6.51)	
Continuous	336 (10.71)	272 (11.78)	64 (7.72)	
Missing	19 (0.61)	10 (0.43)	9 (1.09)	
Child's, n (%)				0.003
Male	1583 (50.45)	1202 (52.06)	381 (45.96)	
Female	1555 (49.55)	1107 (47.94)	448 (54.04)	
Gestational age, n (%)				<0.0001
Term (≥37 weeks)	2448 (78.01)	1739 (75.31)	709 (85.52)	
Late preterm (34-36 weeks)	306 (9.75)	242 (10.48)	64 (7.72)	
Early preterm (<34 weeks)	384 (12.24)	328 (14.21)	56 (6.76)	
Birthweight, n (%)				<0.0001
≥2,500 grams	2275 (72.50)	1599 (69.25)	676 (81.54)	
<2,500 grams	863 (27.50)	710 (30.75)	153 (18.46)	

SD, standard deviation

[a]P-values were obtained from χ^2 tests or t-tests; missing values for categorical variables were incorporated into the largest group

[b]Maternal age at time of delivery

[c]Black includes self-reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[d]Type 2 diabetes mellitus and/or gestational diabetes mellitus

[e]Never smokers were defined as mothers with no history of smoking three months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of three months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting three months prior to and throughout pregnancy

Supplemental Table 5-3. Maternal and child characteristics by maternal plasma high-density lipoprotein cholesterol (HDL-C) levels in the Boston Birth Cohort

Maternal and child characteristics	High HDL-C (N=628)[a]	Low HDL-C (N=201)	P-value[b]
ASD, n (%)	64 (10.19)	22 (10.95)	0.760
Maternal age (years), mean (SD)[c]	28.34 (6.57)	28.13 (6.36)	0.682
Nulliparous, n (%)	271 (43.15)	91 (45.27)	0.598
Race or ethnicity, n (%)[d]			0.198
Black	439 (69.90)	146 (72.64)	
White	24 (3.82)	9 (4.48)	
Hispanic	120 (19.11)	36 (17.91)	
Other	45 (7.17)	10 (4.98)	
Maternal education, n (%)			0.096
Below college degree	528 (84.08)	179 (89.05)	
College degree or above	95 (15.13)	21 (10.45)	
Missing (n=6)	5 (0.80)	1 (0.50)	
Maternal BMI, n (%)			
Mean (SD)	26.45 (6.55)	26.97 (6.73)	0.342
Normal weight (<25 kg/m^2)	302 (48.09)	86 (42.79)	0.326
Overweight (25 - <30 kg/m^2)	152 (24.20)	58 (28.86)	
Obese (≥30 kg/m^2)	146 (23.25)	48 (23.88)	
Missing	28 (4.46)	9 (4.48)	
Maternal Diabetes, n (%)[e]			0.056
No diabetes	565 (89.97)	171 (85.07)	
Diabetes	63 (10.03)	30 (14.93)	
Maternal smoking, n (%)[f]			0.305
Never	539 (85.83)	163 (81.09)	
Quit	37 (5.89)	17 (8.46)	
Continuous	46 (7.32)	18 (8.96)	
Missing	6 (0.96)	3 (1.49)	
Child's, n (%)			0.361
Male	283 (45.06)	98 (48.76)	
Female	345 (54.94)	103 (51.24)	
Gestational age, n (%)			0.968
Term (≥37 weeks)	536 (85.35)	173 (86.07)	
Late preterm (34-36 weeks)	49 (7.80)	15 (7.46)	
Early preterm (<34 weeks)	43 (6.86)	13 (6.47)	
Birthweight, n (%)			0.287
≥2,500 grams	507 (80.73)	169 (84.08)	
<2,500 grams	121 (19.27)	32 (15.92)	

SD, standard deviation

[a]Score derived from factor analysis as the average score of the three BCAAs (leucine, isoleucine, and valine)

[b]P-values were obtained from χ^2 tests or t-tests; missing values for categorical variables incorporated with largest sized group

[c]Maternal age at time of delivery

[d]Black includes self-reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[e]Type 2 diabetes mellitus and/or gestational diabetes mellitus

[f]Never smokers were defined as mothers with no history of smoking three months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of three months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting three months prior to and throughout pregnancy

Supplemental Table 5-4. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) – joint effect with maternal high-density lipoprotein (HDL-C)

	Leucine below median		Leucine above median		
Maternal HDL	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL
High HDL	35/336	1.00 (ref)	29/292	0.95 (0.56-1.59)	0.95 (0.56-1.59)
Low HDL	3/77	0.35 (0.10-1.16)	19/124	1.56 (0.85-2.84)	**4.46 (1.27-15.63)**
OR (95% CI) HDL within strata of BCAA		0.35 (0.10-1.16)		1.64 (0.88-3.05)	
Measure of interaction on additive scale: RERI (95% CI) = **0.12 (0.03-0.21); P = 0.010** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **4.71 (1.21-18.28); P = 0.025**					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL
High HDL	39/338	1.00 (ref)	25/290	0.72 (0.43-1.23)	0.72 (0.43-1.23)
Low HDL	2/74	0.21 (0.05-0.90)	20/127	1.43 (0.80-2.57)	**6.73 (1.53-29.68)**
OR (95% CI) HDL within strata of BCAA		**0.21 (0.05-0.90)**		**1.98 (1.06-3.72)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.16 (0.07-0.25); P = <0.001** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **9.30 (1.93-44.96); P = 0.006**					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL
High HDL	34/336	1.00 (ref)	30/292	1.02 (0.61-1.71)	1.02 (0.61-1.71)
Low HDL	2/77	0.24 (0.06-1.01)	20/124	1.67 (0.87-3.19)	**7.21 (1.64-31.79)**
OR (95% CI) HDL within strata of BCAA		0.24 (0.06-1.01)		1.70 (0.91-3.09)	
Measure of interaction on additive scale: RERI (95% CI) = **0.13 (0.05-0.22); P = 0.003** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **7.09 (1.47-34.13); P = 0.015**					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL
High HDL	35/340	1.00 (ref)	29/288	0.98 (0.58-1.64)	0.98 (0.58-1.64)
Low HDL	3/79	0.34 (0.10-1.15)	19/122	1.61 (0.88-2.93)	**4.67 (1.33-16.36)**

OR (95% CI) HDL within strata of BCAA	0.34 (0.10-1.15)	1.65 (0.88-3.09)

Measure of interaction on additive scale: RERI (95% CI) = **0.12 (0.03-0.21); P = 0.009**
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **4.79 (1.23-18.60); P = 0.024**

Note: Low HDL <50 mg/dl

Supplemental Table 5-5. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of male child autism spectrum disorder (ASD) – joint effect with maternal high-density lipoprotein (HDL-C)

	Leucine below median		Leucine above median		
Maternal HDL	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL
High HDL	23/151	1.00 (ref)	23/132	1.17 (0.62-2.21)	1.17 (0.62-2.21)
Low HDL	2/33	0.36 (0.08-1.60)	16/65	1.82 (0.89-3.73)	**5.06 (1.09-23.54)**
OR (95% CI) HDL within strata of BCAA		0.36 (0.08-1.60)		1.55 (0.75-3.18)	
Measure of interaction on additive scale: RERI (95% CI) = **0.16 (0.005-0.32); P = 0.043**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 4.31 (0.82-22.71); P = 0.085					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL
High HDL	25/158	1.00 (ref)	21/125	1.07 (0.57-2.03)	1.07 (0.57-2.03)
Low HDL	1/32	0.17 (0.02-1.32)	17/66	1.85 (0.92-3.71)	**10.78 (1.36-84.92)**
OR (95% CI) HDL within strata of BCAA		0.17 (0.02-1.32)		1.72 (0.83-3.54)	
Measure of interaction on additive scale: RERI (95% CI) = **0.22 (0.07-0.37); P = 0.004**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **10.01 (1.15-86.95); P = 0.037**					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL
High HDL	23/156	1.00 (ref)	23/127	1.28 (0.68-2.41)	1.28 (0.68-2.41)
Low HDL	2/35	0.35 (0.08-1.56)	16/63	1.97 (0.96-4.04)	**5.62 (1.21-26.09)**
OR (95% CI) HDL within strata of BCAA		0.35 (0.08-1.56)		1.54 (0.75-3.18)	
Measure of interaction on additive scale: RERI (95% CI) = **0.16 (0.005-0.32); P = 0.043**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 4.39 (0.83-23.12); P = 0.081					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of HDL
High HDL	23/156	1.00 (ref)	23/127	1.28 (0.68-2.41)	1.28 (0.68-2.41)
Low HDL	2/34	0.36 (0.08-1.61)	16/64	1.93 (0.94-3.95)	**5.33 (1.15-24.79)**

OR (95% CI) HDL within strata of BCAA	0.36 (0.08-1.61)	1.51 (0.73-3.11)
Measure of interaction on additive scale: RERI (95% CI) = 0.16 (-0.001-0.32); P = 0.051		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 4.17 (0.79-21.97); P = 0.092		

Note: Low HDL <50 mg/dl

Supplemental Table 5-6. Adjusted association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) – joint effect with maternal total cholesterol (TC)

	Leucine below median		Leucine above median		
Maternal TC	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC[a]
Low TC	23/275	1.00 (ref)	38/294	1.88 (1.05-3.37)	1.63 (0.94-2.81)
High TC	15/138	1.70 (0.81-3.57)	10/122	1.12 (0.49-2.54)	0.73 (0.32-1.70)
OR (95% CI) TC within strata of BCAA[a]		1.34 (0.67-2.65)		0.60 (0.29-1.25)	
Measure of interaction on additive scale: RERI (95% CI) = -0.09 (-0.17- <-0.001); P = 0.050 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.35 (0.12-1.02); P = 0.053					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC[a]
Low TC	25/271	1.00 (ref)	36/298	1.41 (0.79-2.49)	1.35 (0.79-2.32)
High TC	16/141	1.42 (0.70-2.89)	9/119	0.92 (0.40-2.14)	0.64 (0.27-1.50)
OR (95% CI) TC within strata of BCAA[a]		1.26 (0.65-2.45)		0.60 (0.28-1.28)	
Measure of interaction on additive scale: RERI (95% CI) = -0.06 (-0.15-0.02); P = 0.149 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.46 (0.16-1.35); P = 0.158					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC[a]
Low TC	22/281	1.00 (ref)	39/288	**2.16 (1.20-3.89)**	**1.84 (1.06-3.20)**
High TC	14/132	1.78 (0.83-3.89)	11/128	1.28 (0.57-2.84)	0.79 (0.35-1.82)
OR (95% CI) TC within strata of BCAA[a]		1.40 (0.69-2.83)		0.60 (0.30-1.21)	
Measure of interaction on additive scale: RERI (95% CI) = **-0.09 (-0.18- -0.003); P = 0.043** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **0.33 (0.11-10.97); P = 0.044**					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC[a]
Low TC	23/280	1.00 (ref)	38/289	**1.94 (1.08-3.48)**	1.69 (0.98-2.92)
High TC	15/139	1.71 (0.81-3.57)	10/121	1.14 (0.50-2.60)	0.74 (0.32-1.73)
OR (95% CI) TC within strata of BCAA[a]		1.35 (0.68-2.68)		0.60 (0.29-1.24)	

Measure of interaction on additive scale: RERI (95% CI) = **-0.09 (-0.17- <-0.001); P = 0.049**
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.35 (0.12-1.01); P = 0.052

Note: High total cholesterol ≥240 mg/dl; ORs adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight unless otherwise noted.
[a]Stratified ORs are unadjusted

Supplemental Table 5-7. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) – joint effect with maternal total cholesterol (TC)

	Leucine below median		Leucine above median		
Maternal TC	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC
Low TC	23/275	1.00 (ref)	38/294	1.63 (0.94-2.81)	1.63 (0.94-2.81)
High TC	15/138	1.34 (0.67-2.65)	10/122	0.98 (0.45-2.12)	0.73 (0.32-1.70)
OR (95% CI) TC within strata of BCAA		1.34 (0.67-2.65)		0.60 (0.29-1.25)	
Measure of interaction on additive scale: RERI (95% CI) = -0.07 (-0.16-0.01); P = 0.104 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.45 (0.17-1.23); P = 0.119					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC
Low TC	25/271	1.00 (ref)	36/298	1.35 (0.79-2.32)	1.35 (0.79-2.32)
High TC	16/141	1.26 (0.65-2.45)	9/119	0.81 (0.36-1.78)	0.64 (0.27-1.50)
OR (95% CI) TC within strata of BCAA		1.26 (0.65-2.45)		0.60 (0.28-1.28)	
Measure of interaction on additive scale: RERI (95% CI) = -0.06 (-0.15-0.02); P = 0.134 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.47 (0.17-1.30); P = 0.147					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC
Low TC	22/281	1.00 (ref)	39/288	**1.84 (1.06-3.20)**	**1.84 (1.06-3.20)**
High TC	14/132	1.40 (0.69-2.83)	11/128	1.11 (0.52-2.36)	0.79 (0.35-1.82)
OR (95% CI) TC within strata of BCAA		1.40 (0.69-2.83)		0.60 (0.30-1.21)	
Measure of interaction on additive scale: RERI (95% CI) = -0.08 (-0.16-0.01); P = 0.084 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.43 (0.16-1.16); P = 0.097					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC
Low TC	23/280	1.00 (ref)	38/289	1.69 (0.98-2.92)	1.69 (0.98-2.92)
High TC	15/139	1.35 (0.68-2.68)	10/121	1.01 (0.46-2.19)	0.74 (0.32-1.73)

OR (95% CI) TC within strata of BCAA	1.35 (0.68-2.68)	0.60 (0.29-1.24)

Measure of interaction on additive scale: RERI (95% CI) = -0.07 (-0.16-0.01); P = 0.094
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.44 (0.16-1.20); P = 0.109

Note: High total cholesterol ≥240 mg/dl

Supplemental Table 5-8. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of male child autism spectrum disorder (ASD) – joint effect with maternal total cholesterol (TC)

	Leucine below median		Leucine above median		
Maternal TC	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC
Low TC	15/129	1.00 (ref)	30/141	**2.05 (1.05-4.02)**	**2.05 (1.05-4.02)**
High TC	10/55	1.69 (0.71-4.04)	9/56	1.46 (0.60-3.56)	0.86 (0.32-2.32)
OR (95% CI) TC within strata of BCAA		1.69 (0.71-4.04)		0.71 (0.31-1.61)	

Measure of interaction on additive scale: RERI (95% CI) = -0.12 (-0.28-0.05); P = 0.163
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.42 (0.13-1.39); P = 0.155

	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC
Low TC	15/129	1.00 (ref)	30/141	**2.05 (1.05-4.02)**	**2.05 (1.05-4.02)**
High TC	11/61	1.67 (0.72-3.90)	8/50	1.45 (0.57-3.66)	0.87 (0.32-2.35)
OR (95% CI) TC within strata of BCAA		1.67 (0.72-3.90)		0.70 (0.30-1.66)	

Measure of interaction on additive scale: RERI (95% CI) = -0.12 (-0.28-0.05); P = 0.166
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.42 (0.13-1.41); P = 0.160

	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC
Low TC	15/135	1.00 (ref)	30/135	**2.29 (1.17-4.48)**	**2.29 (1.17-4.48)**
High TC	10/56	1.74 (0.73-4.15)	9/55	1.57 (0.64-3.83)	0.90 (0.33-2.42)
OR (95% CI) TC within strata of BCAA		1.74 (0.73-4.15)		0.68 (0.30-1.56)	

Measure of interaction on additive scale: RERI (95% CI) = -0.13 (-0.29-0.04); P = 0.135
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.39 (0.12-1.30); P = 0.127

	BCAA below median		BCAA above median		

	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of TC
Low TC	15/134	1.00 (ref)	30/136	**2.25 (1.15-4.40)**	**2.25 (1.15-4.40)**
High TC	10/56	1.72 (0.72-4.11)	9/55	1.55 (0.64-3.79)	0.90 (0.33-2.42)
OR (95% CI) TC within strata of BCAA		1.72 (0.72-4.11)		0.69 (0.30-1.57)	
Measure of interaction on additive scale: RERI (95% CI) = -0.12 (-0.29-0.04); P = 0.143 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.40 (0.12-1.33); P = 0.134					

Note: High total cholesterol ≥240 mg/dl

Supplemental Table 5-9. Adjusted association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) – joint effect with maternal low-density lipoprotein (LDL-C)

	Leucine below median		Leucine above median		
Maternal LDL	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL[a]
Low LDL	32/330	1.00 (ref)	42/353	1.37 (0.81-2.30)	1.26 (0.77-2.05)
High LDL	6/83	0.75 (0.28-1.99)	6/63	0.96 (0.37-2.54)	1.35 (0.41-4.41)
OR (95% CI) LDL within strata of BCAA[a]		0.73 (0.29-1.80)		0.78 (0.32-1.92)	
Measure of interaction on additive scale: RERI (95% CI) = -0.01 (-0.11-0.09); P = 0.843 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.94 (0.24-3.61); P = 0.924					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL[a]
Low LDL	35/331	1.00 (ref)	39/352	1.08 (0.64-1.80)	1.05 (0.65-1.71)
High LDL	6/81	0.65 (0.25-1.70)	6/65	0.86 (0.33-2.24)	1.27 (0.39-4.14)
OR (95% CI) LDL within strata of BCAA[a]		0.68 (0.27-1.67)		0.82 (0.33-2.01)	
Measure of interaction on additive scale: RERI (95% CI) = 0.01 (-0.08-0.11); P = 0.789 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.23 (0.33-4.77); P = 0.760					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL[a]
Low LDL	30/336	1.00 (ref)	44/347	1.66 (0.98-2.80)	1.48 (0.91-2.42)
High LDL	6/77	0.93 (0.35-2.48)	6/69	0.95 (0.36-2.51)	1.13 (0.35-3.67)

OR (95% CI) LDL within strata of BCAA[a]		0.86 (0.35-2.15)		0.66 (0.27-1.61)	
Measure of interaction on additive scale: RERI (95% CI) = -0.04 (-0.14-0.06); P = 0.402 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.62 (0.16-2.41); P = 0.490					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL[a]
Low LDL	32/336	1.00 (ref)	42/347	1.42 (0.84-2.38)	1.31 (0.80-2.13)
High LDL	6/83	0.77 (0.29-2.02)	6/63	0.98 (0.37-2.58)	1.35 (0.41-4.41)
OR (95% CI) LDL within strata of BCAA[a]		0.74 (0.30-1.83)		0.76 (0.31-1.88)	
Measure of interaction on additive scale: RERI (95% CI) = -0.01 (-0.11-0.08); P = 0.796 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.90 (0.23-3.49); P = 0.884					

ORs adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight unless otherwise noted.
[a]Stratified ORs are unadjusted
Note: High LDL ≥160 mg/dl

Supplemental Table 5-10. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) – joint effect with maternal low-density lipoprotein (LDL-C)

	Leucine below median		Leucine above median		
Maternal LDL	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL
Low LDL	32/330	1.00 (ref)	42/353	1.26 (0.77-2.05)	1.26 (0.77-2.05)
High LDL	6/83	0.73 (0.29-1.80)	6/63	0.98 (0.39-2.45)	1.35 (0.41-4.41)
OR (95% CI) LDL within strata of BCAA		0.73 (0.29-1.80)		0.78 (0.32-1.92)	
Measure of interaction on additive scale: RERI (95% CI) = 0.001 (-0.10-0.10); P = 0.986 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.07 (0.30-3.86); P = 0.913					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL
Low LDL	35/331	1.00 (ref)	39/352	1.05 (0.65-1.71)	1.05 (0.65-1.71)
High LDL	6/81	0.68 (0.27-1.67)	6/65	0.86 (0.35-2.14)	1.27 (0.39-4.14)
OR (95% CI) LDL within strata of BCAA		0.68 (0.27-1.67)		0.82 (0.33-2.01)	
Measure of interaction on additive scale: RERI (95% CI) = 0.01 (-0.09-0.12); P = 0.800 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.21 (0.34-4.33); P = 0.773					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL
Low LDL	30/336	1.00 (ref)	44/347	1.48 (0.91-2.42)	1.48 (0.91-2.42)
High LDL	6/77	0.86 (0.35-2.15)	6/69	0.97 (0.39-2.43)	1.13 (0.35-3.67)
OR (95% CI) LDL within strata of BCAA		0.86 (0.35-2.15)		0.66 (0.27-1.61)	
Measure of interaction on additive scale: RERI (95% CI) = -0.03 (-0.13-0.07); P = 0.580 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.76 (0.21-2.73); P = 0.675					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL
Low LDL	32/336	1.00 (ref)	42/347	1.31 (0.80-2.13)	1.31 (0.80-2.13)
High LDL	6/83	0.74 (0.30-1.83)	6/63	1.00 (0.40-2.50)	1.35 (0.41-4.41)

OR (95% CI) LDL within strata of BCAA	0.74 (0.30-1.83)	0.76 (0.31-1.88)

Measure of interaction on additive scale: RERI (95% CI) = -0.003 (-0.11-0.10); P = 0.752
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.03 (0.29-3.71); P = 0.961

Note: High LDL ≥160 mg/dl

Supplemental Table 5-11. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of male child autism spectrum disorder (ASD) – joint effect with maternal low-density lipoprotein (LDL-C)

	Leucine below median		Leucine above median		
Maternal LDL	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL
Low LDL	21/149	1.00 (ref)	34/166	1.57 (0.87-2.85)	1.57 (0.87-2.85)
High LDL	4/35	0.79 (0.25-2.46)	5/31	1.17 (0.41-3.39)	1.49 (0.36-6.13)
OR (95% CI) LDL within strata of BCAA		0.79 (0.25-2.46)		0.75 (0.27-2.09)	

Measure of interaction on additive scale: RERI (95% CI) = -0.02 (-0.20-0.17); P = 0.859
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.95 (0.20-4.40); P = 0.947

	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL
Low LDL	22/154	1.00 (ref)	33/161	1.55 (0.86-2.80)	1.55 (0.86-2.80)
High LDL	4/36	0.75 (0.27-2.09)	5/30	1.20 (0.42-3.47)	1.60 (0.39-6.59)
OR (95% CI) LDL within strata of BCAA		0.75 (0.27-2.09)		0.78 (0.28-2.18)	

Measure of interaction on additive scale: RERI (95% CI) = -0.01 (-0.19-0.18); P = 0.945
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.03 (0.22-4.79); P = 0.966

	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL
Low LDL	21/158	1.00 (ref)	34/157	1.80 (0.99-3.27)	1.80 (0.99-3.27)
High LDL	4/33	0.90 (0.29-2.82)	5/33	1.16 (0.40-3.35)	1.29 (0.31-5.32)
OR (95% CI) LDL within strata of BCAA		0.90 (0.29-2.82)		0.65 (0.23-1.80)	

Measure of interaction on additive scale: RERI (95% CI) = -0.05 (-0.24-0.13); P = 0.572
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.72 (0.15-3.33); P = 0.672

	BCAA below median		BCAA above median		

	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of LDL
Low LDL	21/155	1.00 (ref)	34/160	1.72 (0.95-3.12)	1.72 (0.95-3.12)
High LDL	4/35	0.82 (0.26-2.57)	5/31	1.23 (0.42-3.55)	1.49 (0.36-6.13)
OR (95% CI) LDL within strata of BCAA		0.82 (0.26-2.57)		0.71 (0.25-1.99)	

Measure of interaction on additive scale: RERI (95% CI) = -0.03 (-0.22-0.16); P = 0.752
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.87 (0.19-4.02); P = 0.854

Note: High total LDL ≥160 mg/dl

Supplemental Table 5-12. Adjusted association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) – joint effect with maternal non-high-density lipoprotein (non-HDL-C)

	Leucine below median		Leucine above median		
Maternal non-HDL	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL[a]
Low non-HDL	31/324	1.00 (ref)	42/341	1.46 (0.86-2.47)	1.33 (0.81-2.17)
High non-HDL	7/89	0.83 (0.33-2.10)	6/75	0.79 (0.30-2.07)	1.02 (0.33-3.17)
OR (95% CI) non-HDL within strata of BCAA[a]		0.81 (0.34-1.90)		0.62 (0.25-1.51)	
Measure of interaction on additive scale: RERI (95% CI) = -0.04 (-0.13-0.06); P = 0.437 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.65 (0.18-2.41); P = 0.523					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL[a]
Low non-HDL	34/326	1.00 (ref)	39/339	1.15 (0.68-1.93)	1.12 (0.69-1.82)
High non-HDL	7/86	0.72 (0.29-1.81)	6/78	0.69 (0.27-1.81)	0.94 (0.30-2.93)
OR (95% CI) non-HDL within strata of BCAA[a]		0.76 (0.33-1.78)		0.64 (0.26-1.57)	
Measure of interaction on additive scale: RERI (95% CI) = -0.01 (-0.11-0.08); P = 0.751 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.84 (0.23-3.08); P = 0.789					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL[a]
Low non-HDL	29/329	1.00 (ref)	44/336	**1.72 (1.02-2.93)**	1.56 (0.95-2.56)
High non-HDL	7/84	0.94 (0.37-2.40)	6/80	0.83 (0.32-2.18)	0.89 (0.29-2.78)
OR (95% CI) non-HDL within strata of BCAA[a]		0.94 (0.40-2.23)		0.54 (0.22-1.31)	
Measure of interaction on additive scale: RERI (95% CI) = -0.05 (-0.15-0.04); P = 0.292 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.52 (0.14-1.92); P = 0.239					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL[a]
Low non-HDL	31/331	1.00 (ref)	42/334	1.52 (0.90-2.56)	1.39 (0.85-2.27)
High non-HDL	7/88	0.85 (0.34-2.14)	6/76	0.80 (0.30-2.09)	0.99 (0.32-3.09)

OR (95% CI) non-HDL within strata of BCAA[a]	0.84 (0.36-1.97)	0.60 (0.24-1.46)

Measure of interaction on additive scale: RERI (95% CI) = -0.04 (-0.13-0.05); P = 0.385
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.62 (0.17-2.29); P = 0.474

ORs adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight unless otherwise noted.
[a]Stratified ORs are unadjusted
Note: High non-HDL ≥190 mg/dl

Supplemental Table 5-13. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of child autism spectrum disorder (ASD) – joint effect with maternal non-high-density lipoprotein (non-HDL-C)

	Leucine below median		Leucine above median		
Maternal non-HDL	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL
Low non-HDL	31/324	1.00 (ref)	42/341	1.33 (0.81-2.17)	1.33 (0.81-2.17)
High non-HDL	7/89	0.81 (0.34-1.90)	6/75	0.83 (0.33-2.05)	1.02 (0.33-3.17)
OR (95% CI) non-HDL within strata of BCAA		0.81 (0.34-1.90)		0.62 (0.25-1.51)	
Measure of interaction on additive scale: RERI (95% CI) = -0.03 (-0.12-0.07); P = 0.592 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.77 (0.22-2.65); P = 0.675					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL
Low non-HDL	34/326	1.00 (ref)	39/339	1.12 (0.69-1.82)	1.12 (0.69-1.82)
High non-HDL	7/86	0.76 (0.33-1.78)	6/78	0.72 (0.29-1.77)	0.94 (0.30-2.93)
OR (95% CI) non-HDL within strata of BCAA		0.76 (0.33-1.78)		0.64 (0.26-1.57)	
Measure of interaction on additive scale: RERI (95% CI) = -0.02 (-0.11-0.08); P = 0.754 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.84 (0.24-2.90); P = 0.786					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL
Low non-HDL	29/329	1.00 (ref)	44/336	1.56 (0.95-2.56)	1.56 (0.95-2.56)
High non-HDL	7/84	0.94 (0.40-2.23)	6/80	0.84 (0.34-2.09)	0.89 (0.29-2.78)
OR (95% CI) non-HDL within strata of BCAA		0.94 (0.40-2.23)		0.54 (0.22-1.31)	
Measure of interaction on additive scale: RERI (95% CI) = -0.05 (-0.15-0.04); P = 0.292 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.57 (0.17-1.98); P = 0.377					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL
Low non-HDL	31/331	1.00 (ref)	42/334	1.39 (0.85-2.27)	1.39 (0.85-2.27)
High non-HDL	7/88	0.84 (0.36-1.97)	6/76	0.83 (0.33-2.06)	0.99 (0.32-3.09)

OR (95% CI) non-HDL within strata of BCAA	0.84 (0.36-1.97)	0.60 (0.24-1.46)

Measure of interaction on additive scale: RERI (95% CI) = -0.03 (-0.13-0.06); P = 0.502
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.71 (0.21-2.46); P = 0.592

ORs adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight unless otherwise noted.
Note: High non-HDL ≥190 mg/dl

Supplemental Table 5-14. Crude association of maternal plasma branched-chain amino acids (BCAAs) and risk of male child autism spectrum disorder (ASD) – joint effect with maternal non-high-density lipoprotein (non-HDL-C)

	Leucine below median		Leucine above median		
Maternal non-HDL	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL
Low non-HDL	20/147	1.00 (ref)	34/160	1.71 (0.94-3.14)	1.71 (0.94-3.14)
High non-HDL	5/37	0.99 (0.35-2.85)	5/37	0.99 (0.35-2.85)	1.00 (0.26-3.79)
OR (95% CI) non-HDL within strata of BCAA		0.99 (0.35-2.85)		0.58 (0.21-1.60)	
Measure of interaction on additive scale: RERI (95% CI) = -0.08 (-0.25-0.10); P = 0.397 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.58 (0.14-2.52); P = 0.471					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL
Low non-HDL	21/152	1.00 (ref)	33/155	1.69 (0.93-3.07)	1.69 (0.93-3.07)
High non-HDL	5/38	0.95 (0.33-2.69)	5/36	1.01 (0.35-2.88)	1.06 (0.28-4.04)
OR (95% CI) non-HDL within strata of BCAA		0.95 (0.33-2.69)		0.60 (0.22-1.65)	
Measure of interaction on additive scale: RERI (95% CI) = -0.07 (-0.24-0.11); P = 0.456 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.63 (0.15-2.72); P = 0.537					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL
Low non-HDL	20/153	1.00 (ref)	34/154	**1.88 (1.03-3.45)**	**1.88 (1.03-3.45)**
High non-HDL	5/38	1.01 (0.35-2.88)	5/36	1.07 (0.37-3.08)	1.06 (0.28-4.04)
OR (95% CI) non-HDL within strata of BCAA		1.01 (0.35-2.88)		0.57 (0.21-1.58)	
Measure of interaction on additive scale: RERI (95% CI) = -0.08 (-0.26-0.09); P = 0.360 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.56 (0.13-2.44); P = 0.445					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of non-HDL
Low non-HDL	20/153	1.00 (ref)	34/154	**1.88 (1.03-3.45)**	**1.88 (1.03-3.45)**
High non-HDL	5/37	1.04 (0.36-2.98)	5/37	1.04 (0.36-2.98)	1.00 (0.26-3.79)

OR (95% CI) non-HDL within strata of BCAA	1.04 (0.36-2.98)	0.55 (0.20-1.52)

Measure of interaction on additive scale: RERI (95% CI) = -0.09 (-0.28-0.09); P = 0.319
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.53 (0.12-2.29); P = 0.396

Note: High non-HDL ≥190 mg/dl

CHAPTER 6 MATERNAL MULTIPLE METABOLIC DISORDERS, PLASMA BRANCHED-CHAIN AMINO ACIDS, AND THE RISK OF CHILD AUTISM SPECTRUM DISORDER: EVIDENCE OF SEX DIFFERENCE

ABSTRACT

Background

Obesity and diabetes are known risk factors for ASD and are associated with branched-chain amino acids (BCAAs). However, the association of maternal multiple metabolic disorders (MMD) alone or in combination with BCAAs, with child ASD has not been well-studied. Bringing several lines of research under a life course framework, we examined joint associations of maternal MMD and BCAAs on child ASD using a prospective birth cohort design.

Methods

We analyzed 829 mother-infant pairs (including 86 ASD) from the Boston Birth Cohort. Based on pre-pregnancy overweight or obesity, type 2 or gestational diabetes, high-density lipoprotein cholesterol (HDL<50mg/dl), and hypertensive disorders, mothers were grouped into MMD (score$\geq$3) or no MMD (score<3). Liquid chromatography-tandem mass spectrometry was used to quantify maternal postpartum plasma BCAAs and factor analysis was used to create a composite BCAA score. Logistic regression was used to examine additive and interactive associations of maternal MMD and BCAAs on child ASD risk, adjusting for pertinent covariates. Differential associations by child's sex were also examined.

Results

The median composite MMD score was 1 (IQR: 0-2). Mothers with an MMD score ≥3 were at a significantly greater risk for having a child with ASD (crude OR: 2.03 95% CI: 1.11-3.71), though this significance was lost upon adjustment with pertinent variables. Maternal high MMD score and high BCAA score synergistically increased the risk of ASD (adjusted OR: 3.20, 95% CI: 1.65-6.18; p for interaction= 0.019) and the association was more pronounced among male children (crude OR: 4.53, 95% CI: 2.02-10.16). The highest ASD risk was among children with all three risk factors – male sex, high MMD score, and high BCAA score (crude OR: 12.20, 95% CI: 4.89-30.46) compared to children without any of these factors. A similar, yet weaker, association was observed among children with other developmental disorders (adjusted OR: 3.30, 95% CI: 1.78-6.12).

Conclusion

Maternal MMD and elevated plasma BCAAs synergistically increased the child risk of ASD, especially among males, providing new evidence on multiple early life maternal metabolic factors on child ASD risk and early life origin of sex difference in ASD.

INTRODUCTION

Autism spectrum disorders (ASDs) are defined by core abnormalities in social interaction and communication and by the presence of restricted or repetitive interests and behaviors.[1] The causes of ASD remains largely unknown, but both genetics and the environment are believed to contribute to its etiology.[2]

We and others have shown maternal obesity and diabetes are associated with child risk of ASD.[3-5] It is well-observed that in a given individual, multiple metabolic disorders tend to co-exist. For example, metabolic syndrome is a loosely defined term for co-occurring cardio-metabolic conditions,[6] and typically comprises elevated blood pressure, dyslipidemia, hyperglycemia, and central obesity. Metabolic syndrome is highly prevalent among adults in countries with Western diets.[7-9] Among US women, the prevalence of metabolic syndrome was close to 35% between 2007-2012, up from 26% during 1988-1994.[10] The role of maternal multiple metabolic disorders in child ASD risk has not been well-studied and is worth investigation. For example, the prevalence of pre-pregnancy hypertension was significantly greater among mothers who had children with ASD, and together with obesity and diabetes, pre-pregnancy hypertension was associated with a higher likelihood of having a child with ASD.[11] Given the well-observed co-occurring of multiple cardiometabolic disorders in the population, it would be important to consider both individual components as well as multiple metabolic disorders (MMD) as a whole in relation to ASD.

Leucine, isoleucine, and valine are essential amino acids that constitute the branched-chain amino acids (BCAAs). These key macronutrients make up about a third of muscle protein and are also important cellular signaling molecules.[12] Higher circulating concentrations of BCAAs

are known to be associated with diabetes, in particular, and are predictive of incident type 2 diabetes.[13] A few studies have also reported associations between BCAAs and individuals with ASD in children; however, the studies were most cross-sectional in design and findings were mostly inconsistent.[14-16] Given the increasing prevalence of MMD among women of reproductive age and the association of their individual components with BCAAs and ASD, there is a strong scientific premise to investigate whether maternal MMD and BCAAs can jointly increase child risk of ASD.

However, there is a lack of studies on the prospective association between maternal MMD, BCAAs and child risk of ASD. In this study, we brought together several lines of research to address these important questions using a prospective birth cohort design. Three key aspects of this research are addressed in this study: the role of maternal MMD in child's risk of ASD; whether maternal MMD and BCAAs additively or interactively increase the child risk of ASD; and whether the associations differ by child's, given the striking sex difference observed in ASD prevalence.

METHODS

Participants and Data Collection

The Boston Birth Cohort (BBC) is an ongoing prospective birth cohort study, recruiting mothers from the Boston Medical Center since 1998. The current analysis is based on data from this dataset between 2004-2017. Starting from 2004, children were followed from birth, with a mean follow-up period of 9.1 years. Briefly, mothers were approached 24-72 hours post-partum and informed consent was obtained upon enrolment. Additional recruitment details have been previously published.[3] Exclusion criteria included multiple deliveries, pregnancies due to *in vitro*

fertilization, and babies born with major birth defects or chromosomal abnormalities. The Johns Hopkins Bloomberg School of Public Health and the Boston University Medical Center Institutional Review Boards (IRB) reviewed and approved the initial and follow-up studies.

Of 3,163 mothers enrolled in the Boston Birth Cohort (BBC), 829 mother-infant pairs were included in this study. Maternal and infant medical records were reviewed and abstracted and mothers were interviewed in person using a standardized questionnaire. Follow-up information on each child was obtained through child medical records as well as maternal interviews during well-child visits. Maternal blood was collected during enrolment in a non-fasted state and fractionated at the Boston Medical Center. Quantitative profiling of maternal plasma metabolites was conducted at the Harvard-Massachusetts Institute of Technology (MIT) Broad Institute Metabolite Profiling Laboratory using liquid chromatography tandem mass spectrometry (LC-MS/MS). The present study was limited to mothers who had metabolite measurements and no missing covariates (Supplemental Figure 6-1).

Outcome

While there was no systematic screening for ASD, all children were evaluated by highly trained medical staff at the Boston Medical Center autism evaluation program who communicate regularly with the medical center primary care physicians. ASD was defined using ICD-9 and ICD-10 primary and secondary diagnoses from electronic medical records (EMR) (Table 3-3). Children who were ever diagnosed with autism (299.00), Asperger syndrome (299.80), and/or pervasive developmental disorder - not otherwise specified (299.90) were defined as ASD cases. Children without ASD but with attention deficit hyperactivity disorder (ADHD), intellectual disabilities, or developmental delays were classified as having other developmental disorders and

were excluded from the present analysis. Children without ASD or other developmental disorders were classified as typically developing (TD).

Exposures

Metabolic syndrome is defined by at least three criteria of the following: hyperglycemia or insulin resistance, obesity or high waist circumference, dyslipidemia (high triglycerides and low HDL), and hypertension.[17] Based on availability of data and previous findings from the BBC, we developed a multiple metabolic disorders (MMD) score based on the following:

- Pre-gestational overweight (body mass index ≥ 25 and <30 kg/m^2) – *1 point*
  OR pre-gestational obesity (BMI ≥30 kg/m^2) – *2 points*
- Gestational diabetes mellitus (GDM; ICD-9 codes: 648.00 or 648.03) – *1 point*
  OR pre-gestational type 2 diabetes (ICD-9 codes: 250.00–250.93) – *2 points*
- Hypertensive disorder (from EMR) (any one of the following: eclampsia, pre-eclampsia, chronic hypertension, gestational hypertension, or hemolysis elevated liver enzymes low platelet count (HELLP) syndrome)[18] – *1 point*
- HDL-C <50 mg/dl – *1 point*

In considering the functional relationship between the MMD score and ASD risk (Figure 6-1), in the subsequent analyses, we grouped subjects into a MMD (score ≥3) vs no MMD (score <3).

Covariates

Maternal covariates included age at delivery; race-ethnicity (black, white, Hispanic, or other); smoking during pregnancy ("never smoked," "ever smoked," or "continuous smoking" three months prior to conception); parity (nulliparous vs multiparous), and education ("high school or less" vs "some college or more"). Child covariates included child's sex (female vs male); and

gestational age and birthweight (categorized into four groups: 1. full term (≥37 completed weeks of gestation) and non-low birthweight (non-LBW; ≥2500g); 2. full term and LBW; 3. preterm and non-LBW; and 4. preterm and LBW).

Statistical analysis

Maternal and child demographic and clinical characteristics were compared by ASD case status t-tests and chi-squared tests for continuous and categorical variables, respectively. Missing values were incorporated with the largest category for main covariates. Undetected metabolite values were imputed with one-half the limit of detection. Metabolite levels were inverse-normally transformed to produce standardized distributions. Factor analysis on the three BCAAs was conducted to create a BCAA score using the Anderson Rubin Method.[19]

Multivariate logistic regression was used to explore the relationship of all the factors included in our criteria for MMD with ASD. We then explored the joint associations of the BCAAs with MMD. For this analysis, the reference category was defined as children whose mothers did not have MMD and had below median concentrations of BCAAs. This group was compared to the three other combinations with the doubly-exposed group being children whose mothers had both MMD and above median concentrations of BCAAs. Additive interaction was assessed by relative excess risk due to interaction (RERI).[20] Multiplicative interaction was also assessed. All analyses were conducted using Stata v14.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Of 3,163 mothers enrolled in the Boston Birth Cohort (BBC), 829 mother-infant pairs from a predominantly urban, low-income, minority population were included in the present study. Maternal and child characteristics by ASD case status have been previously published for this

population and are thus included as a supplementary table with an added comparison of maternal hypertensive disorders (Supplemental Table 6-1). Mothers with ASD children were older ($p=0.01$) and more obese ($p=0.02$) than mothers with TD children. Apart from BMI and a marginally significant difference in diabetes ($p=0.05$), no other MMD component was significantly different between these groups of mothers. More ASD children were male ($p<0.0001$) and had shorter gestation ($p<0.0001$) and lower birthweight ($p=0.04$) compared to TD children. Maternal and child characteristics for included and excluded participants are compared in (Supplemental Table 6-2).

Univariate logistic regression analysis revealed maternal obesity and type 2 diabetes as significantly associated with child ASD risk, while the other MMD components were not associated (Table 6-1). Maternal obesity remained significant after adjusting for potential confounders (adjusted odds ratio (aOR): 1.77, 95% CI: 1.02-3.08). When analyzed categorically, the odds ratio for three or more MMD components on the risk of child ASD was significant (OR: 2.03, 95% CI: 1.11-3.71), however this association lost significance when adjusted for potential confounders.

Compared to children born to mothers without MMD and with below median BCAA concentrations, children born to mothers with MMD and above median BCAA concentrations were significantly more at risk of developing ASD (BCAA score aOR: 2.87, 95% CI: 1.40 – 5.89) (Table 6-2). This was true for each individual BCAA. There was also evidence of additive and multiplicative interactions for these associations (BCAA score p for additive interaction = 0.02; p for multiplicative interaction = 0.03). This association was greater among male children than the overall effect (crude BCAA score aOR: 4.53, 95% CI: 2.02-10.16), while no effect was observed among females (Table 6-3). There was also significant additive interaction for the

association among males (p for interaction =0.02). The greatest effect was observed for the group of males whose mothers had MMD and above median BCAA concentrations compared to the reference group: females with mothers without MMD and with below median BCAA concentrations (crude OR: 12.20, 95% CI: 4.89-30.46). Compared to obesity and/or diabetes alone, additional components of the MMD add considerably to this risk (Supplemental Figure 6-2).

Maternal BCAAs alone were not associated with other developmental disorders (DD) (data not shown), and MMD was significantly associated with other DD ($p<0.01$, data not shown) however, it lost significance upon adjustment of key variables. Joint associations were observed between maternal MMD and BCAAs on the risk of other DD (BCAA score OR 1.77, 95% CI 1.16, 2.70, p for interaction = 0.03) and was reflective of all three BCAAs (Supplemental Table 6-3). As found in ASD, there was a sex difference observed for other DD as well (Supplemental Tables 6-4 - 6-5), with males at a higher risk than females (BCAA score OR 2.39 95% CI 1.35, 4.22). Significant interactions were observed for leucine and isoleucine. The risk for other DD was the highest when all three risk factors were combined – male sex, maternal MMD, and above median maternal BCAAs – adjusted for key covariates (Supplemental Figure 6-3).

DISCUSSION

Main findings

Several components of maternal MMD have been implicated in the pathophysiology of ASD, including obesity and waist circumference, insulin resistance, and hypertension. However, prior studies were mostly focused on one or a few components of MMD, thus were unable to examine their joint effects on ASD. This study has simultaneously considered four maternal metabolic

disorders: overweight or obesity, gestational diabetes/diabetes, low HDL, and hypertensive disorders. We found that MMD was indeed significantly associated with risk of ASD in our unadjusted analyses, but not after adjustment with potential confounders. More importantly, we revealed that MMD and BCAAs had a significant synergistic joint effect on child risk of ASD after adjusting for relevant covariates. When stratified by sex of the child, the synergistic effect was most pronounced among males and was insignificant in females. With all three risk factors combined – male child, elevated maternal BCAAs, and maternal MMD – the risk of ASD was the greatest. A similar trend was observed for children with other DD, however, the effect was not as strong.

Interpretation

There is evidence that components of MMD often co-exist and influence each other and are thus difficult to tease apart. Obesity and large waist circumference are predictors of diabetes and cardiovascular disease,[21,22] and dyslipidemia may lead to gestational diabetes and pre-eclampsia during pregnancy.[23] In turn, insulin may alter cholesterol metabolism and biosynthesis in trophoblast cells of obese women.[24] Further still, maternal preeclampsia is associated with increases in cholesterol in both mother and fetus.[25,26]

Maternal MMD components are characterized by oxidative stress and inflammation. As with acute infections, in these chronic conditions, inflammatory mediators are known to cross the placenta during pregnancy.[27] Growing evidence links maternal immune activation (MIA) to child ASD and it is suggested this is due to epigenetic changes.[28] For example, oxidative stress induced by metabolic conditions like diabetes and high cholesterol results in the accumulation of free radicals, including reactive oxygen species (ROS),[29,30] which can alter DNA methylation of

the fetus.[31,32] Obesity can also alter the transcriptome of the placenta during early gestation, leading to energy imbalances and mitochondrial dysfunction.[24,33] One theory posits the combination of genetics and environmental risk factors causing oxidative stress in early life give rise to schizophrenia.[34] Myelin, for example, is highly susceptible to oxidative stress and required for optimal brain development and function.[34] Abnormal myelin formation is also characteristic of ASD.[35] Furthermore, elevated BCAA concentrations also increase ROS via activation of the mammalian target of rapamycin (mTOR) signaling pathway.[36] This can lead to abnormal beta oxidation and potentially mitochondrial dysfunction, and is thought to be linked to ASD etiology.[37,38] Thus, it is possible the effects of maternal oxidative stress paired with the genetic makeup of the fetus could lead to the development of ASD. Epigenetic changes are also seen in cases of preterm birth, a condition highly associated with ASD.[39,40] There is evidence of significantly reduced mRNA levels of brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) – both implicated in ASD etiology – in cord plasma of preterm neonates compared to their full term counterparts.[41] Because the etiology of ASD has been associated with both genetic and environmental risk factors, epigenetic changes during fetal development are an important area of scientific inquiry.[42]

Though we cannot directly identify the proximal source of high levels of maternal plasma BCAAs, we do know they are associated with the mothers' metabolic condition.[13,43] It is possible that excess protein consumption may be a contributing factor, and it has been shown that reducing dietary BCAA intake is associated with lower circulating levels.[44] Red meat and poultry, specifically, are consistently reported to be associated with obesity, inflammation, and diabetes.[44-46] In a study with over 38,000 subjects, high intake of animal protein was associated

with a two-fold increased risk of incident type 2 diabetes.[47] Another study reported subjects who underwent a six-week pescatarian fast had reduced levels of all three BCAAs compared to their baseline.[44] Valine levels were reduced by almost 20% at the end of the six-week period. A randomized crossover control study showed that within 48 hours, a vegan diet not only reduced levels of total BCAAs, it significantly reduced insulin, HOMA-IR, triglycerides, and total cholesterol to HDL-C ratio.[48] Several animal studies have also shed light on chemical and physiological effects of reducing dietary BCAAs, including decreased repetitive self-grooming behavior and mTOR activity, improved glucose tolerance, and rapid fat loss in mice.[49,50] Furthermore, growing pigs fed an optimal BCAA ratio (Leu:Ile:Val = 1:0.75:0.75 to 1:0.25:0.25) within a restricted protein diet exhibited a shift from fatty acid synthesis to fatty acid oxidation in the liver compared to the control group.[51] Taken together, these reports highlight the positive effects of a reduced BCAA diet and call for additional dietary intervention studies in subjects with MMD components.

Strengths and Limitations

Our study leveraged a relatively large intergenerational prospective birth cohort. Maternal circulating BCAAs were quantified using metabolomic profiling. It is the first to evaluate the joint effects of MMD with maternal metabolites on child risk of ASD. More importantly, this study brings forth potential maternal biomarkers of child ASD in concert with maternal MMD and the male fetus. Our study also consisted of an urban, low-income minority group, often under-represented in ASD research.

The primary limitation of our study was that the blood draw was conducted at one time-point post-delivery and taken in a non-fasted state. Maternal lipids and BCAA metabolites were

measured from the plasma. The peri-partum period is a time of metabolic flux and mothers may experience shifts in protein and hormone homeostasis. There may also be changes in medications during this time. It is unknown how this peri-partum period may have affected circulating amino acid concentrations and our observed associations between maternal BCAAs and child risk of ASD. Another limitation was due to the enrolment period for the BBC spanning across the transition from the American Psychiatric Association's Diagnostic and Statistical Manual fourth edition (DSM-IV) to the fifth edition (DSM-5) and ICD-9 to ICD-10. The diagnostic criteria for ASD were redefined, which may have resulted in inconsistencies in diagnoses across the two periods. Our study findings may not be generalizable to the larger US population since the study participants came from a predominantly urban, low-income, minority population. Though our study is the largest metabolomics study of its kind, the sample size was insufficient for detailed stratified analysis. Therefore, we view our findings as hypothesis-generating and call for further research in this area.

Implications for future research

Most previous studies on BCAAs in ASD have been in ASD individuals using a cross-sectional design and report decreased concentrations compared to TD individuals.[15,16] However, our research, conducted in mothers, reports the opposite. Though this finding awaits confirmation of future studies, we posit that this dissonance may be explained by the developmental origins of health and disease model.[52] BCAAs along with other amino acids are transferred across the placental barrier to provide nutrients to the growing fetus and lower levels of BCAA uptake were observed in pregnancies complicated by intrauterine growth restriction.[53] BCAA uptake, in particular, was reported to be significantly lower in babies born small for gestational age compared to normal birthweight babies. Gürke et al., 2015 found that rabbit embryos of dams

with insulin resistance and elevated BCAA levels had a two-fold increase in BCAA concentrations as well as increased mTOR activity.[54]

Therefore, if the fetus is exposed to elevated concentrations of BCAAs, it is likely to read it as a signal of excess in the postnatal environment and thus be programmed to lower its BCAA metabolism. Other possible explanations of lower BCAA concentrations among individuals with ASD include, mutations in the branched chain ketoacid dehydrogenase kinase (BCKDK),[55] a key enzyme in BCAA metabolism, poor diet quality as a cause of sensitivities to certain tastes and textures,[56] and abnormal gut flora that can produce BCAAs *de novo*, as an altered gut microbiome has been associated with ASD.[57]

Future studies may consider performing measurements of MMD components and plasma at several timepoints, including preconception and throughout pregnancy as well as plasma from cord blood and during early childhood. Comparing maternal and cord metabolites may help fill the gaps in our knowledge. If our findings are confirmed, BCAAs may serve as potential early biomarkers in high risk pregnancies with maternal MMD and a male fetus. One study reported over 90% sensitivity and specificity in predicting specific metabotypes of ASD using biomarkers; thus, identifying maternal metabotypes may be a useful next step in early risk prevention.[58] Finally, this study focused on BCAAs while other metabolites remain to be explored.

Conclusion

In this longitudinal prospective birth cohort study, we found that maternal MMD was associated with child risk of ASD. Maternal MMD also had joint and synergistic effects with maternal plasma BCAA concentrations, and when stratified by sex, these effects only remained in males.

Our findings are considered preliminary and hypotheses generating and warrant further studies with larger sample size and additional timepoints to clarify the role of maternal MMD, maternal BCAAs, and sex differences in the pathway from *in-utero* metabolic environment to child postnatal development of ASD.

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Table 6-1. Individual components of maternal multiple metabolic disorders (MMD) and composite MMD score, N (% ASD) = 829 (10.4%)

Metabolic Syndrome Components[a]	Crude OR (95% CI)	Adjusted OR[b] (95% CI)
Diabetes		
No Diabetes	1.00 (ref)	1.00 (ref)
GDM	1.39 (0.47-4.08)	1.06 (0.33-3.37)
T2DM	**2.02 (1.01-4.05)**	1.73 (0.83-3.62)
Obesity		
Normal weight	1.00 (ref)	1.00 (ref)
Overweight	0.92 (0.50-1.68)	0.74 (0.39-1.42)
Obese	**1.86 (1.12-3.08)**	**1.77 (1.02-3.08)**
HDL <50 mg/dl	1.08 (0.65-1.81)	1.05 (0.61-1.80)
Hypertension	1.01 (0.54-1.89)	0.70 (0.35-1.39)
MMD Score		
0	1.00 (ref)	1.00 (ref)
1	0.91 (0.48-1.71)	0.71 (0.36-1.38)
2	1.01 (0.53-1.93)	1.04 (0.52-2.08)
3+	**2.03 (1.11-3.71)**	1.47 (0.75-2.86)
Test for trend[c]	p = 0.215	p = 0.268

[a]Univariate logistic regression
[b]ORs adjusted for maternal age, race/ethnicity, education, parity, smoking status, and child's, gestational age, and birthweight
[c]Likelihood ratio test for trend

Table 6-2. Adjusted joint association of maternal multiple metabolic disorders (MMD) and branched-chain amino acids (BCAAs) with risk of child autism spectrum disorder (ASD)

	Leucine below median		Leucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	30/335	1.00 (ref)	31/347	0.95 (0.55-1.65)	1.00 (0.59-1.69)
MMD	8/78	0.77 (0.32-1.88)	17/69	**2.85 (1.39-5.86)**	**2.86 (1.15-7.13)**
OR (95% CI) MMD within strata of BCAA*		1.16 (0.51-2.64)		**3.33 (1.72-6.45)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.14 (0.03-0.25); P = 0.012**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **3.87 (1.25-11.57); P = 0.019**					
	Isoleucine below median		Isoleucine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	33/343	1.00 (ref)	28/339	0.81 (0.46-1.41)	0.85 (0.50-1.43)
MMD	8/69	0.81 (0.33-1.97)	17/78	**2.24 (1.11-4.53)**	2.13 (0.85-5.29)
OR (95% CI) MMD within strata of BCAA*		1.23 (0.54-2.80)		**3.10 (1.60-6.00)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.12 (0.02-0.23); P = 0.025**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **3.41 (1.11-6.93); P = 0.033**					
	Valine below median		Valine above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	28/337	1.00 (ref)	33/345	1.16 (0.66-2.01)	1.17 (0.69-1.98)
MMD	8/76	0.87 (0.36-2.13)	17/71	**3.09 (1.50-6.39)**	**2.68 (1.07-6.67)**
OR (95% CI) MMD within strata of BCAA*		1.30 (0.57-2.97)		**2.98 (1.55-5.72)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.12 (0.01-0.23); P = 0.029**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 3.08 (0.99-9.48); P = 0.050					
	BCAA below median		BCAA above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	30/342	1.00 (ref)	31/340	1.02 (0.59-1.78)	1.04 (0.62-1.77)
MMD	8/77	0.82 (0.34-1.99)	17/70	**2.87 (1.40-5.89)**	**2.77 (1.11-6.90)**

OR (95% CI) MMD within strata of BCAA*	1.21 (0.53-2.74)	**3.20 (1.65-6.18)**
Measure of interaction on additive scale: RERI (95% CI) = **0.13 (0.02-0.24); P = 0.019**		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **3.45 (1.12-10.63); P = 0.031**		

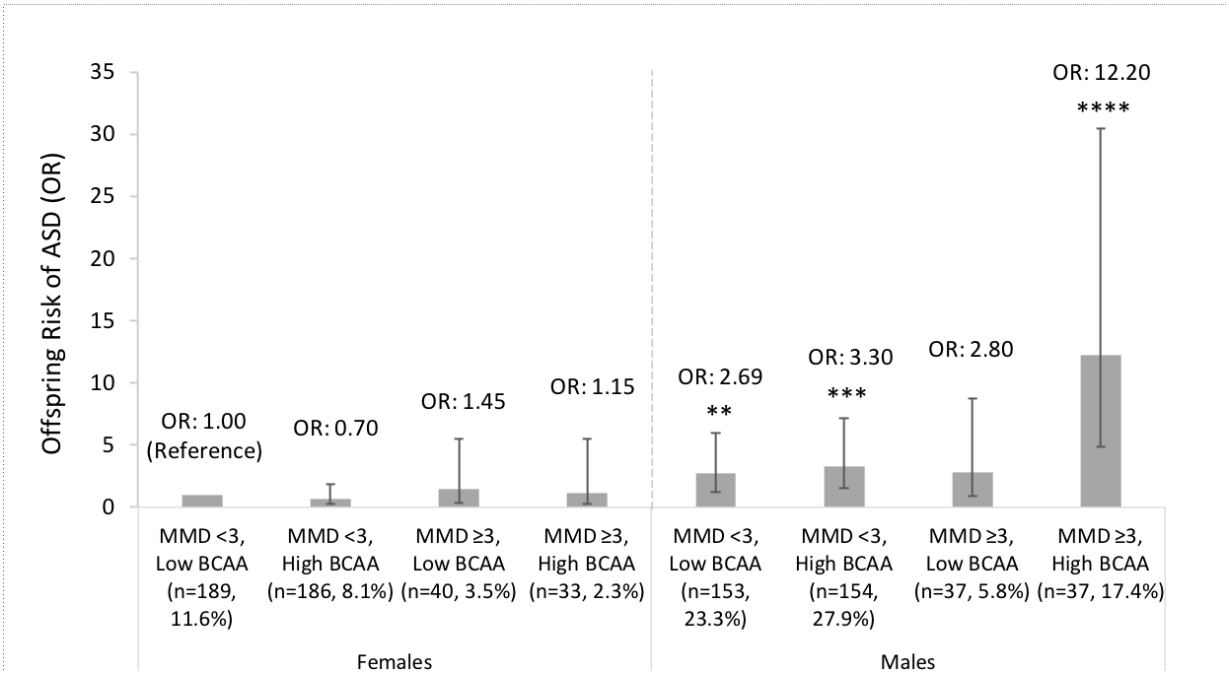
RERI Relative excess risk due to interaction; maternal metabolic syndrome defined as a score of ≥3
Note: ORs adjusted for maternal age, race/ethnicity, education, parity, smoking status, and gestational age/birthweight unless otherwise noted. *Stratified ORs unadjusted

Table 6-3. Crude joint association of maternal multiple metabolic disorders (MMD) and branched-chain amino acids (BCAAs) with risk of child autism spectrum disorder (ASD) among male and female children

	Males				
	BCAA score below median		BCAA score above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD
No MMD	30/342	1.00 (ref)	31/340	1.23 (0.65-2.33)	1.23 (0.65-2.33)
MMD	8/77	1.04 (0.36-2.98)	17/70	**4.53 (2.02-10.16)**	**4.36 (1.38-13.76)**
OR (95% CI) MMD within strata of BCAA		1.04 (0.36-2.98)		**3.69 (1.68-8.12)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.25 (0.04-0.45); P = 0.021** Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 3.55 (0.95-13.24); P = 0.059					
	Females				
	BCAA score below median		BCAA score above median		
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD
No MMD	10/189	1.00 (ref)	7/186	0.70 (0.26-1.88)	0.70 (0.26-1.88)
MMD	3/40	1.45 (0.38-5.53)	2/33	1.15 (0.24-5.53)	0.80 (0.12-5.07)
OR (95% CI) MMD within strata of BCAA		1.45 (0.38-5.53)		1.65 (0.33-8.31)	
Measure of interaction on additive scale: RERI (95% CI) = 0.001 (-0.12-0.12); P = 0.989 Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.14 (0.14-9.27); P = 0.905					

RERI Relative excess risk due to interaction
Maternal metabolic syndrome defined as a score of ≥3

Figure 6-1. Joint association of maternal multiple metabolic disorders (MMD), BCAA score, and child's sex on the risk of child autism spectrum disorder (ASD)



(n, % ASD); *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ***p ≤ 0.0001,
Maternal multiple metabolic disorders defined as a score of ≥3

Supplemental Table 6-1. Maternal and child characteristics by child autism spectrum disorder status (typically developing (TD) vs. ASD) in the Boston Birth Cohort

Maternal Characteristics	Total (N=829)	TD (N=743)	ASD (N=86)	P-value[a]
Age (years), n (%)[b]				
Mean (SD)	28.29 (6.51)	28.09 (6.51)	30.00 (6.34)	0.010
≤25	298 (35.95)	274 (36.88)	24 (27.91)	0.257
26-35	390 (47.04)	344 (46.30)	46 (53.49)	
≥36	141 (17.01)	125 (16.82)	16 (18.60)	
Nulliparous, n (%)	362 (43.67)	327 (44.01)	35 (40.70)	0.344
Race or ethnicity, n (%)[c]				0.089
Black	585 (70.57)	533 (71.74)	52 (60.47)	
White	33 (3.98)	28 (3.77)	5 (5.81)	
Hispanic	156 (18.82)	132 (17.77)	24 (27.91)	
Other	55 (6.63)	50 (6.73)	5 (5.81)	
Education, n (%)				0.991
Below college degree	707 (85.28)	635 (85.46)	72 (83.72)	
College degree or above	116 (13.99)	104 (14.00)	12 (13.95)	
Missing	6 (0.72)	4 (0.54)	2 (2.33)	
Pre-pregnancy BMI, n (%)				
Mean (SD)	26.57 (6.59)	26.41 (6.42)	27.96 (7.91)	0.046
Normal weight (<25 kg/m^2)	388 (46.80)	354 (47.64)	34 (39.53)	0.019
Overweight (25 - <30 kg/m^2)	210 (25.33)	193 (25.98)	17 (19.77)	
Obese (≥30 kg/m^2)	194 (23.40)	165 (22.21)	29 (33.72)	
Missing	37 (4.46)	31 (4.17)	6 (6.98)	
Diabetes, n (%)[d]				0.053
No diabetes	736 (88.78)	665 (89.50)	71 (82.56)	
Diabetes	93 (10.76)	78 (10.50)	15 (17.44)	
Smoking, n (%)[e]				0.665
Never	702 (84.68)	632 (85.06)	70 (81.40)	
Quit	54 (6.51)	47 (6.33)	7 (8.14)	
Continuous	64 (7.72)	56 (7.54)	8 (9.30)	
Missing	9 (1.09)	8 (1.08)	1 (1.16)	
HDL cholesterol, mean (SD)	62.77 (17.98)	63.08 (18.26)	60.02 (15.16)	0.135
Low HDL (<50 mg/dl), n (%)	201 (24.25)	179 (24.09)	22 (25.58)	0.760
Hypertensive disorders, n (%)[f]	124 (14.96)	111 (14.94)	13 (15.12)	0.965
Leucine (above median), n (%)	416 (50.18)	368 (49.53)	48 (55.81)	0.270
Isoleucine (above median), n (%)	417 (50.30)	372 (50.07)	45 (52.33)	0.692
Valine (above median), n (%)	416 (50.18)	366 (49.26)	50 (58.14)	0.119
BCAA score (above median), n (%)	410 (49.46)	362 (48.72)	48 (55.81)	0.213
Child Characteristics	Total (N=829)	TD (N=743)	ASD (N=86)	P-value[a]
Sex, n (%)				<0.0001
Male	381 (45.96)	317 (42.66)	64 (74.42)	
Female	448 (54.04)	426 (57.34)	22 (25.58)	
Gestational age, n (%)				<0.0001
Term (≥37 weeks)	709 (85.52)	645 (86.81)	64 (74.42)	
Late preterm (34-36 weeks)	64 (7.72)	60 (8.08)	4 (4.65)	
Early preterm (<34 weeks)	56 (6.76)	38 (5.11)	18 (20.93)	
Birth weight, n (%)				0.036
≥2,500 grams	676 (81.54)	613 (82.50)	63 (73.26)	

<2,500 grams	153 (18.46)	130 (17.50)	23 (26.74)

SD, standard deviation; LDL, low-density lipoprotein; HDL, high-density lipoprotein

[a]P-values were obtained from χ^2 tests or t-tests; missing values for categorical variables were incorporated into the largest group

[b]Maternal age at time of delivery

[c]Black includes self- reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[d]Type 2 diabetes mellitus and/or gestational diabetes mellitus

[e]Never smokers were defined as mothers with no history of smoking six months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of six months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting six months prior to and throughout pregnancy

[f]Hypertension defined by any one of the following: eclampsia, pre-eclampsia, chronic hypertension, gestational hypertension, or hemolysis elevated liver enzymes low platelet count (HELLP) syndrome

Supplemental Table 6-2. Maternal and child characteristics of Boston Birth Cohort participants excluded and included in the analysis

Characteristics	Total, N (%)	Excluded, N (%)	Included, N (%)	P-value[a]
Total	3138 (100.00)	2309 (73.58)	829 (26.42)	
Maternal age (years), mean (SD)[b]	28.64 (6.50)	28.60 (6.48)	28.29 (6.51)	0.234
Nulliparous, n (%)	1337 (42.61)	975 (42.23)	362 (43.67)	0.472
Race or ethnicity, n (%)[c]				<0.0001
Black	1998 (63.67)	1413 (61.20)	585 (70.57)	
White	227 (7.23)	194 (8.40)	33 (3.98)	
Hispanic	701 (22.34)	545 (23.60)	156 (18.82)	
Other	212 (6.76)	156 (6.80)	55 (6.63)	
Maternal education, n (%)				0.683
Below college degree	2690 (85.72)	1983 (85.88)	707 (85.28)	
College degree or above	426 (13.58)	310 (13.43)	116 (13.99)	
Missing	22 (0.70)	16 (0.69)	6 (0.72)	
Maternal BMI, n (%)				
Mean (SD)	26.58 (6.65)	26.59 (6.67)	26.57 (6.59)	0.950
Normal weight (<25 kg/m^2)	1452 (46.27)	1064 (46.08)	388 (46.80)	0.898
Overweight (25 - <30 kg/m^2)	813 (25.90)	603 (26.12)	210 (25.33)	
Obese (≥30 kg/m^2)	703 (22.40)	509 (22.04)	194 (23.40)	
Missing	170 (5.42)	133 (5.76)	37 (4.46)	
Maternal Diabetes, n (%)[d]				0.255
No diabetes	2751 (87.67)	2015 (87.27)	736 (88.78)	
Diabetes	387 (12.33)	294 (12.73)	93 (11.22)	
Maternal smoking, n (%)[e]				
Never	2542 (81.01)	1840 (79.69)	702 (84.68)	0.001
Quit	241 (7.68)	187 (8.10)	54 (6.51)	
Continuous	336 (10.71)	272 (11.78)	64 (7.72)	
Missing	19 (0.61)	10 (0.43)	9 (1.09)	
Child's, n (%)				0.003
Male	1583 (50.45)	1202 (52.06)	381 (45.96)	
Female	1555 (49.55)	1107 (47.94)	448 (54.04)	
Gestational age, n (%)				<0.0001
Term (≥37 weeks)	2448 (78.01)	1739 (75.31)	709 (85.52)	
Late preterm (34-36 weeks)	306 (9.75)	242 (10.48)	64 (7.72)	
Early preterm (<34 weeks)	384 (12.24)	328 (14.21)	56 (6.76)	
Birthweight, n (%)				<0.0001
≥2,500 grams	2275 (72.50)	1599 (69.25)	676 (81.54)	
<2,500 grams	863 (27.50)	710 (30.75)	153 (18.46)	

SD, standard deviation

[a]P-values were obtained from χ^2 tests or t-tests; missing values for categorical variables were incorporated into the largest group

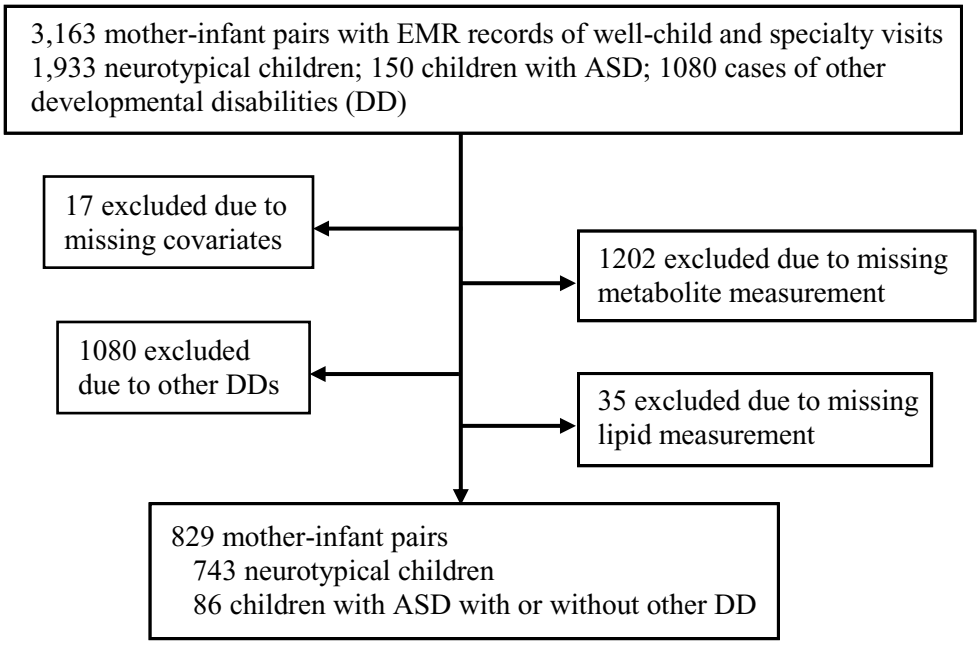
[b]Maternal age at time of delivery

[c]Black includes self-reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races

[d]Type 2 diabetes mellitus and/or gestational diabetes mellitus

[e]Never smokers were defined as mothers with no history of smoking three months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of three months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting three months prior to and throughout pregnancy

Supplemental Figure 6-1. Flowchart of study sample included and excluded in the analyses



Supplemental Table 6-3. Adjusted joint association of maternal multiple metabolic disorders (MMD) and branched-chain amino acids (BCAAs) with risk of other child developmental disorders (Other DD)

	Leucine below median		Leucine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD
No MMD	209/518	1.00 (ref)	50/122	0.97 (0.75-1.26)	0.98 (0.75-1.28)
MMD	193/505	0.93 (0.61-1.42)	69/119	**1.88 (1.23-2.88)**	**1.93 (1.12-3.33)**
OR (95% CI) MMD within strata of BCAA		0.88 (0.57-1.37)		**2.00 (1.30-3.06)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.19 (0.05-0.33); P = 0.007**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **2.09 (1.15-3.78); P = 0.015**					
	Isoleucine below median		Isoleucine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD
No MMD	219/528	1.00 (ref)	45/106	0.87 (0.67-1.14)	0.87 (0.67-1.14)
MMD	183/495	0.90 (0.58-1.41)	74/135	**1.61 (1.08-2.41)**	**1.75 (1.01-3.03)**
OR (95% CI) MMD within strata of BCAA		0.84 (0.53-1.33)		**1.88 (1.25-2.81)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.17 (0.03-0.31); P = 0.018**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **2.05 (1.13-3.72); P = 0.018**					
	Valine below median		Valine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD
No MMD	215/522	1.00 (ref)	52/120	0.89 (0.69-1.16)	0.90 (0.69-1.17)
MMD	187/501	0.98 (0.64-1.50)	67/121	**1.62 (1.06-2.47)**	1.62 (0.94-2.79)
OR (95% CI) MMD within strata of BCAA		0.96 (0.62-1.48)		**1.85 (1.21-2.83)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.16 (0.02-0.30); P = 0.025**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **1.85 (1.03-3.35); P = 0.041**					
	BCAA below median		BCAA above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD
No MMD	209/521	1.00 (ref)	51/120	0.98 (0.75-1.27)	0.99 (0.76-1.28)

MMD	193/502	0.99 (0.65-1.51)	68/121	**1.77 (1.16-2.70)**	**1.76 (1.02-3.01)**
OR (95% CI) MMD within strata of BCAA		0.94 (0.61-1.45)		**1.87 (1.22-2.86)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.15 (0.02-0.29); P = 0.030**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **1.83 (1.02-3.32); P = 0.044**					

RERI: Relative excess risk due to interaction; No MMD: score <3; MMD: score ≥3
Note: ORs adjusted for maternal age, race/ethnicity, education, parity, smoking status, and gestational age/birthweight

Supplemental Table 6-4. Adjusted joint association of maternal multiple metabolic disorders (MMD) and branched-chain amino acids (BCAAs) with risk of other child developmental disorders (Other DD), among males

	Leucine below median		Leucine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	130/258	1.00 (ref)	29/61	1.27 (0.74-2.19)	0.77 (0.54-1.09)
MMD	105/240	1.33 (0.99-1.78)	36/58	**2.45 (1.39-4.32)**	1.81 (0.87-3.75)
OR (95% CI) MMD within strata of BCAA*		0.89 (0.51-1.56)		**2.10 (1.17-3.79)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.21 (0.01-0.41); P = 0.036**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.28 (0.98-5.31); P = 0.055					
	Isoleucine below median		Isoleucine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	140/273	1.00 (ref)	28/58	1.27 (0.73-2.21)	**0.69 (0.49-0.99)**
MMD	95/225	1.21 (0.90-1.64)	37/61	**2.27 (1.31-3.92)**	1.65 (0.80-3.42)
OR (95% CI) MMD within strata of BCAA*		0.89 (0.50-1.56)		**2.11 (1.18-3.76)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.21 (0.02-0.41); P = 0.034**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **2.43 (1.05-5.65); P = 0.038**					
	Valine below median		Valine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	141/275	1.00 (ref)	32/64	1.40 (0.83-2.37)	**0.69 (0.49-0.99)**
MMD	94/223	1.19 (0.88-1.62)	33/55	**2.14 (1.20-3.81)**	1.50 (0.72-3.11)
OR (95% CI) MMD within strata of BCAA*		0.95 (0.55-1.64)		**2.06 (1.13-3.76)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.19 (-0.01-0.39); P = 0.059					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.10 (0.90-4.88); P = 0.085					
	BCAA below median		BCAA above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	131/264	1.00 (ref)	30/62	1.33 (0.78-2.27)	0.81 (0.57-1.16)
MMD	104/234	**1.37 (1.02-1.85)**	35/57	**2.39 (1.35-4.22)**	1.70 (0.82-3.52)

OR (95% CI) MMD within strata of BCAA*	0.95 (0.55-1.67)	**1.99 (1.10-3.60)**
Measure of interaction on additive scale: RERI (95% CI) = 0.18 (-0.02-0.38); P = 0.071		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.02 (0.87-4.65); P = 0.102		

RERI: Relative excess risk due to interaction; No MMD: score <3; MMD: score ≥3

Note: ORs adjusted for maternal age, race/ethnicity, education, parity, smoking status, and gestational age/birthweight unless otherwise noted *Stratified ORs unadjusted

Supplemental Table 6-5. Adjusted joint association of maternal multiple metabolic disorders (MMD) and branched-chain amino acids (BCAAs) with risk of other child developmental disorders (Other DD), among females

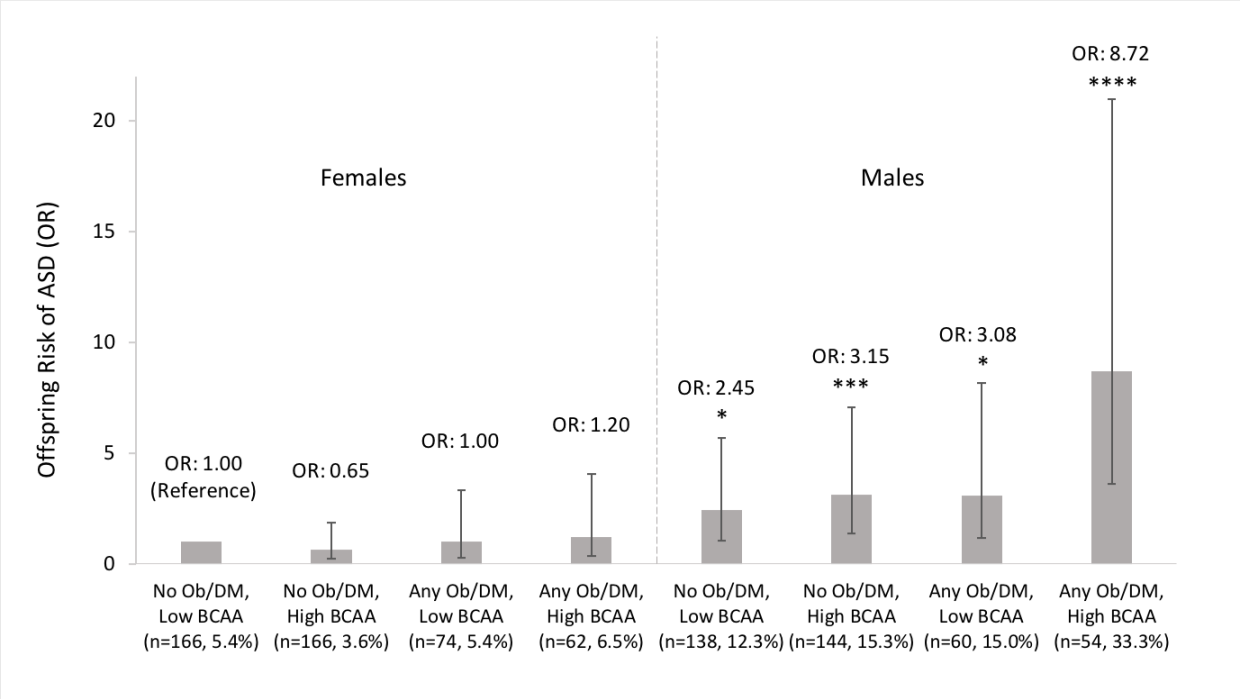
	Leucine below median		Leucine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	79/260	1.00 (ref)	21/61	0.63 (0.36-1.10)	1.14 (0.79-1.65)
MMD	88/265	**0.67 (0.50-0.90)**	33/61	1.34 (0.78-2.30)	**2.24 (1.08-4.66)**
OR (95% CI) MMD within strata of BCAA*		1.20 (0.67-2.17)		**2.37 (1.35-4.17)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.17 (-0.02-0.36); P = 0.083					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.77 (0.76-4.11); P = 0.188					
	Isoleucine below median		Isoleucine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	79/255	1.00 (ref)	17/48	0.65 (0.35-1.21)	1.08 (0.75-1.56)
MMD	88/270	**0.63 (0.47-0.84)**	37/74	1.13 (0.69-1.86)	1.82 (0.86-3.85)
OR (95% CI) MMD within strata of BCAA*		1.22 (0.64-2.34)		**2.07 (1.23-3.49)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.13 (-0.06-0.32); P = 0.190					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.66 (0.70-3.93); P = 0.250					
	Valine below median		Valine above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	74/247	1.00 (ref)	20/56	0.67 (0.37-1.20)	1.18 (0.81-1.70)
MMD	93/278	**0.65 (0.48-0.87)**	34/66	1.18 (0.70-1.99)	1.91 (0.92-3.97)
OR (95% CI) MMD within strata of BCAA*		1.30 (0.71-2.39)		**2.11 (1.23-3.64)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.12 (-0.07-0.31); P = 0.208					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.49 (0.64-3.50); P = 0.355					
	BCAA below median		BCAA above median		
	N Other DD/ Total	OR (95% CI)	N Other DD/ Total	OR (95% CI)	OR (95% CI) BCAA within strata of MMD*
No MMD	78/257	1.00 (ref)	21/58	0.68 (0.38-1.19)	1.14 (0.79-1.65)
MMD	89/268	**0.65 (0.48-0.87)**	33/64	1.20 (0.70-2.04)	1.88 (0.91-3.88)

OR (95% CI) MMD within strata of BCAA*	1.30 (0.72-2.37)	**2.14 (1.23-3.72)**
Measure of interaction on additive scale: RERI (95% CI) = 0.12 (-0.07-0.32); P = 0.201		
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.52 (0.66-3.53); P = 0.328		

RERI: Relative excess risk due to interaction; No MMD: score <3; MMD: score ≥3

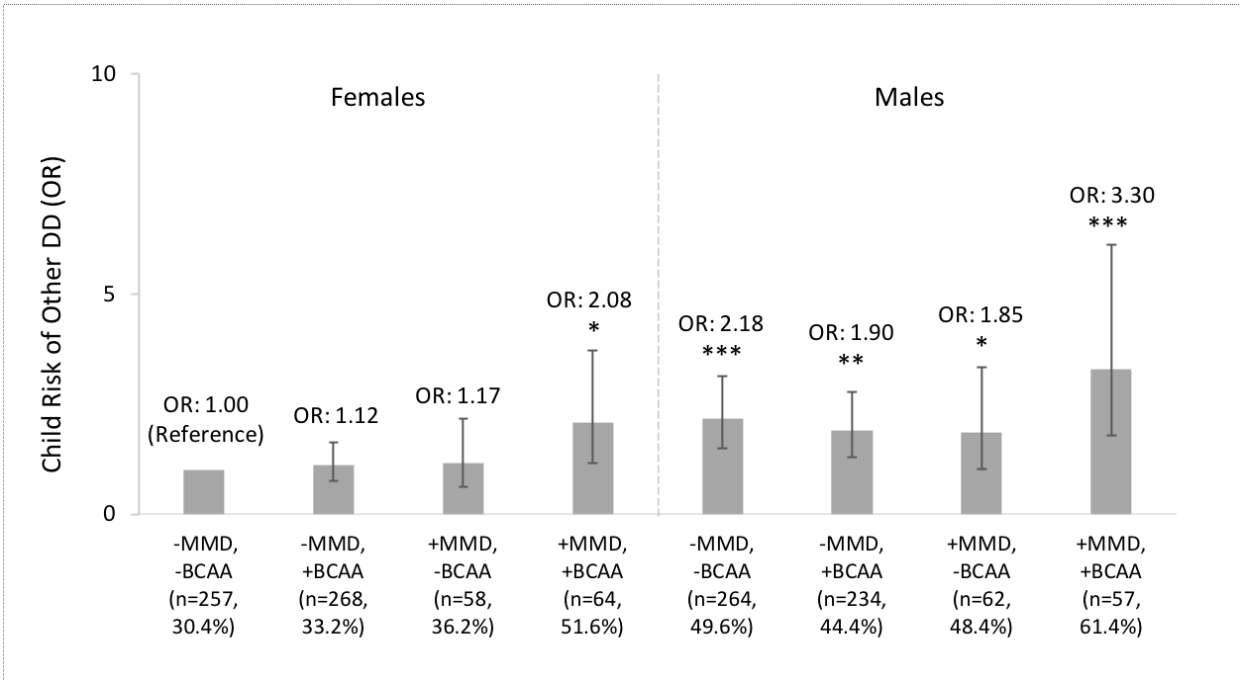
Note: ORs adjusted for maternal age, race/ethnicity, education, parity, smoking status, and gestational age/birthweight unless otherwise noted *Stratified ORs unadjusted

Supplemental Figure 6-2. Joint association of maternal obesity/diabetes (ob/DM), BCAA score, and child's sex on the risk of child autism spectrum disorders (ASD)



(n, % ASD); *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001
-MMD: Maternal multiple metabolic disorders with a score of <3
+MMD: Maternal multiple metabolic disorders with a score of ≥3
-BCAA: Below median maternal plasma branched-chain amino acid concentrations
+BCAA: Above median maternal plasma branched-chain amino acid concentrations

Supplemental Figure 6-3. Joint association of maternal obesity/diabetes (ob/DM), BCAA score, and child's sex on the risk of child other developmental disorders (other DD)



(n, % other DD); *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001
-MMD: Maternal multiple metabolic disorders with a score of <3
+MMD: Maternal multiple metabolic disorders with a score of ≥3
-BCAA: Below median maternal plasma branched-chain amino acid concentrations
+BCAA: Above median maternal plasma branched-chain amino acid concentrations

CHAPTER 7 DISCUSSION AND RESEARCH IMPLICATIONS

Maternal obesity and diabetes are known risk factors for autism spectrum disorders (ASD). This research was motivated by the potential for understanding mechanistic pathways underlying the associations between maternal metabolic disorders and child risk of ASD employing targeted metabolomics. In this dissertation, the associations between maternal branched-chain amino acids (BCAA; leucine, isoleucine, and valine) metabolites and child's risk of ASD as well their joint effect with maternal metabolic conditions, including obesity/diabetes (ob/DM), dyslipidemia, and multiple metabolic disorders (MMD) were examined within a prospective birth cohort study design. This chapter summarizes the key findings of this research, its strengths and limitations, its implications, and directions for future research.

SUMMARY OF KEY FINDINGS

Aim 1 sought to explore the role of maternal plasma BCAAs in the pathway from maternal obesity/diabetes to child ASD. Maternal BCAAs were not found to be associated with child risk of ASD and thus did not mediate the path between maternal ob/DM and child ASD. However, when elevated, all the BCAAs had synergistic associations with maternal ob/DM after being adjusted for pertinent covariates (BCAA score OR: 2.35; 95% CI: 1.21, 4.55). Leucine and isoleucine had significant interactions on the additive scale and isoleucine on the multiplicative scale. The combination of maternal ob/DM and low BCAA concentrations was not associated with child risk of ASD, highlighting the strong influence of BCAA concentrations in mothers with ob/DM. The BCAAs also had joint effects with the child's sex on the risk of ASD (crude BCAA score OR: 4.9; 95% CI: 1 2.48, 9.69). With all three risk factors – maternal obesity,

maternal elevated BCAAs, and male sex – the effect was the greatest (crude BCAA score OR: 8.72; 95% CI: 3.62, 20.99). This effect was also observed for other DD, though to a lesser extent.

Aim 2 assessed the association between maternal cholesterol and child ASD and explore the role of maternal plasma BCAAs in the pathway from maternal dyslipidemia to child ASD. Maternal plasma cholesterols (total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and non-HDL-C) were not associated with child risk of ASD. Low maternal HDL-C concentrations compounded the male child's risk of ASD (OR: 4.20; 95% CI: 2.02, 8.73), though the interaction was not statistically significant. Among mothers with low HDL-C, elevated BCAAs further increased the risk of child ASD (BCAA score OR: 4.67; 95% CI: 1.33, 16.36; p for interaction: 0.006). Compared to all other combinations, males with elevated maternal BCAAs and low HDL-C had the highest risk of ASD (crude OR: 4.78, 95% CI: 2.12, 10.78). Results for non-HDL-C and LDL-C cholesterols were not as robust as those for HDL.

Aim 3 assessed the association between maternal multiple metabolic disorders (MMD) and child ASD and explore the role of maternal plasma BCAAs in the pathway from maternal MMD to child ASD. We found that maternal MMD (composite score ≥3, based on: 1 point for overweight or 2 points obesity; 1 point for gestational diabetes or 2 points for diabetes; 1 point for low HDL; 1 point for hypertensive disorders) was associated with child risk of ASD and had joint additive interaction with maternal plasma BCAA concentrations. When stratified by child's, this association remained only in males. The greatest effect was observed for males whose mothers had MMD and above median BCAA concentrations compared to females whose mothers did not have MMD and had below median BCAA concentrations (crude OR: 12.20, 95% CI: 4.89-30.46). This pattern was seen among children with other DD as well, though the effect was not as

strong. Compared to obesity and/or diabetes alone or HDL-C alone, all the components of MMD together contribute to an increased risk.

STRENGTHS AND LIMITATIONS

Strengths

Ours is the first prospective birth cohort study to examine the association of maternal metabolites with child risk of ASD. Metabolomics is a comprehensive approach that allows for specific analysis of targeted metabolites. The metabolites in this study were targeted based on known associations with metabolic conditions like obesity and diabetes. The diagnoses of all ASD cases were obtained from electronic medical records after having been evaluated by highly trained staff at the Boston Medical Center, who are in direct contact with primary care physicians.

We leveraged the large Boston Birth Cohort (BBC), a longitudinal and intergenerational study. Because of the intergenerational design of the study, we were able to examine temporal relationships between maternal and child risk factors during pregnancy and the outcome of child ASD. Additionally, as this time period is within the critical window of neurodevelopment, it can provide insight on the causal pathways between maternal metabolic conditions, maternal BCAAs, child's, and risk of child ASD. The BBC study population consisted of an urban, low-income, minority population within the Boston area, where the risk of metabolic conditions like obesity and diabetes is higher than the national average.[1] Thus, we had greater power to examine the potential effects of these conditions on the risk of ASD. Furthermore, we studied an under-represented population in the literature, adding to the current knowledge in ASD research.

The population also included female cases of ASD, providing us with the ability to investigate sex interactions with risk factors of interest. This is the first study, to our knowledge, to show

maternal metabolic conditions, including obesity, diabetes, and low HDL-C, are more harmful to male children than female children in terms of ASD risk. Furthermore, it is also the first to assess the associations between maternal BCAA concentrations and ASD, as well as joint effects of maternal BCAAs and metabolic conditions on ASD. We are also adding to the limited literature on the intergenerational effects of maternal cholesterol levels and maternal multiple metabolic disorders on child risk of ASD.

Limitations

Our study also had limitations. The measurement of maternal plasma lipids and metabolites were only measured at one time-point postpartum. During the peri-partum period, hormonal and physiological changes have an effect on circulating metabolite concentrations, though it is unclear how BCAA concentrations may have been affected or to what extent it affected our observed associations with child risk of ASD. Additional measurements throughout pregnancy and from the child cord blood and early life would have provided a clearer picture of the relationships between maternal BCAAs and child risk of ASD as well as a temporal association between maternal cholesterols and child risk of ASD. Blood was collected in a non-fasted state, which affected our cholesterol measurements, except for HDL-C. Measurements for total cholesterol and triglycerides tend to be inflated during the non-fasted state and may bias the results towards the null. Therefore, our study focused mainly on the associations with HDL-C.

The BBC enrolment spanned across the transition from the American Psychiatric Association's Diagnostic and Statistical Manual fourth edition (DSM-IV) to the fifth edition (DSM-5) and from ICD-9 to ICD-10. The definition of ASD changed during this time, so there may be inconsistencies in diagnoses between these two periods.

Though our study is the largest prospective study of its kind, a larger sample size would have allowed for adjusted sex-specific and subtype analyses. Additionally, because our participants represented an urban, low-income, minority population, our study findings may not be generalizable to the larger ASD community. Thus, our research may be taken as hypothesis-generating, and future studies with larger numbers of individuals with ASD are needed to confirm our findings.

PUBLIC HEALTH IMPLICATIONS AND FUTURE RESEARCH

At present, there is no accurate way to predict a newborn's future risk of ASD and there is no cure for ASD. The mechanisms behind the sex difference in ASD are still unknown. Current treatments take on behavioral and pharmaceutical approaches;[2] however, there are no treatments that have shown to improve the core symptoms of social communication or restricted interests and stereotyped behaviors, though a few recent studies have shown promise.[3,4] Primary prevention would significantly improve the quality of life for many individuals and families members who would otherwise have been affected, and it would further greatly reduce the economic burden associated with ASD.

This research brought forth insights on the early life origins of ASD, specifically surrounding the mechanisms from maternal metabolic disorders to child ASD risk. It also shed light on how BCAAs and child's sex play key roles given these maternal conditions. This was a novel study designed to explore the intergenerational relationship between maternal BCAAs and child ASD risk. It was also the first to find that elevated BCAA score further increased the risk of mothers with ob/DM, HDL-C, or MMD and that these risks were highest among male children.

BCAAs are essential amino acids that make up the highest percentage of amino acids in most animal-sourced proteins.[5] Several studies have pointed to the benefits of lowering BCAA in the diet in both animals and humans as reducing dietary proteins have been associated with circulating BCAA levels.[6] Animal proteins, specifically red meat and poultry have been associated with inflammation, obesity, and diabetes.[6-8] However, BCAAs are necessary building blocks of our muscles and have important roles to support cell growth and maintenance.[5] It is perhaps in excess amounts they may be harmful as they are highly associated with obesity and diabetes according to several sources.[5,9,10] Though our investigation was exploratory and our findings hypothesis generating, if confirmed, they could lead to clinical or public health guidelines for lower BCAA diets in pregnant women at higher risk for a child with ASD, i.e., having a metabolic condition and a male fetus.

Obesity and diabetes are recognized risk factors for child ASD risk.[11-13] However, my research shows the possibility of low HDL levels, together with elevated BCAA concentrations, may increase the risk of child ASD as well, especially among males. MMD revealed an even greater risk. Thus, the guidelines for HDL cholesterol levels during pregnancy should be reviewed and possibly set to a higher cut-point than the overall recommended level set by the American Heart Association (>50 mg/dL) if our finding is confirmed.[14] Metabolic conditions arise due to many factors, including genetics. However, diet and exercise are key drivers in their pathophysiology and should be top priority to women who are pregnant or planning to become pregnant.[15]

There is a disproportionate prevalence of ASD in males compared to females, however the cause remains elusive.[16] Our findings suggest that sexual dimorphism in ASD is influenced by maternal metabolic conditions together with maternal plasma BCAA concentrations and thus may begin *in utero*. Authors of a recent study reported sex-specific differences in BCAA

concentrations in second trimester amniotic fluid.[17] A prevailing theory behind the sex difference in ASD is that higher concentrations of estradiol in the womb protect the female fetal brain, while male fetuses are left vulnerable to inflammatory insults.[18,19] Our findings give credence to this theory as estradiol has an anti-inflammatory effect during neurodevelopment. Under inflammatory conditions brought about by maternal metabolic disorders and elevated BCAAs, estradiol may be protecting female fetuses from inflammatory markers.

Additional studies with a larger, nationally representative sample are required to confirm our findings. These studies would benefit from measurements at multiple time points in both mothers and children, particularly from cord blood. Investigating other metabolites in this manner would provide additional information that may better help fit the pieces together.

Metabolic disorders, especially obesity and diabetes, are inflammatory conditions. Thus, it can be theorized that pregnant women or women planning to become pregnant with these conditions may have a chance of lowering their risk of having a child with ASD by reducing the inflammation and oxidative stress in their bodies. Anti-inflammatory diets have shown some potential in treating children with ASD. These have included gluten-free and casein-free diets, along with dietary supplements.[20] However, in a recent review of the literature, dietary therapies were not found to have significant impacts on the symptoms of ASD.[20] With small sample sizes and inadequate study designs, the evidence remains inconclusive.

In a double-blind randomized controlled trial (RCT), researchers from Harvard and Johns Hopkins Medical Schools treated the symptoms of ASD using sulforaphane, an isothiocyanate having high antioxidant properties, extracted from broccoli sprouts and also found clinically significant improvements in core features.[4] One intervention study has since replicated the basic findings of this study and there are four others currently underway.[21] Though these studies have

only been carried out in individuals with ASD, there is potential for antioxidant treatment in expectant mothers. Sulforaphane and moringin, its counterpart from the subtropical moringa plant, have also been shown to alleviate symptoms of diabetes and prevent cancer via its anti-inflammatory properties.[22,23] Thus, there is pharmacological potential in these plant components for prevention of ASD risk in children by supplementing mothers with metabolic conditions before or during pregnancy.

CONCLUSIONS

The prevalence of ASD has been rapidly increasing over the past few decades and now affects 1 in 59 children in the United States.[24] ASD can be highly disabling and imposes a huge burden to the individuals, their families, and societies, as well as an enormous economic cost. The exact cause of the disorder is still unclear, as its etiology is complex and treatment methods are limited at best. Maternal metabolic conditions, including obesity and diabetes, are known risk factors for ASD. This dissertation sought to investigate the underlying mechanisms between maternal metabolic disorders and child ASD. We found that when paired with elevated maternal BCAA concentrations, ob/DM, low HDL-C, and MMD each further increased the risk of child ASD, and these risks were consistently higher among male children. Additional studies are required to confirm these findings and identify potential early life interventions for the primary prevention of ASD.

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CURRICULUM VITAE

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EDUCATION

2019 PhD, Human Nutrition, Department of International Health, The Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD
Thesis: Joint Effects of Maternal Metabolic Conditions and Plasma Branched-Chain Amino Acids on the Risk of Child Autism Spectrum Disorder: Evidence of Sex Difference
GPA: 3.92

2012 MPH, Nutrition, Hubert Department of Global Health, Emory University, Rollins School of Public Health, Atlanta, GA
Thesis: Impact of the Family Health Program in Vespasiano, Brazil on the Nutritional Status of Children Five Years and Younger
GPA: 3.65

2009 BA, Neurobiological Sciences, Global Health Minor, Northwestern University, Weinberg School of Arts and Sciences, Evanston, IL
GPA: 3.46

PROFESSIONAL EXPERIENCE

2018 – 2019 Research Assistant, Center on the Early Life Origins of Disease, Department of Population, Family and Reproductive Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Responsibilities: Analyzing associations between the maternal plasma metabolome and subsequent diagnosis of autism spectrum disorder in offspring

2016 – 2019 Research Assistant, Lewis B. and Dorothy Cullman Chemoprotection Center, Johns Hopkins School of Medicine, Baltimore, MD
Responsibilities: Conducting research on phytochemicals including quantifying concentrations and preparing dietary interventions for clinical studies

2016 – 2017 Research Assistant, Center for Human Nutrition, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Responsibilities: Analyzed data from the Nepal Nutrition Intervention Project to examine the association between maternal inflammation and child growth

2015 – 2017 Research Assistant, Institute of International Programs, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Responsibilities: Reviewed literature and conducted meta-analyses to identify preventive interventions for stunting and wasting and published as first author

2013 – 2015 Statistical Analyst/Programmer, Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL
Responsibilities: Developed and executed programs for analysis of multiple projects to study prevention of chronic diseases using a variety of statistical techniques, interpreted analyses and prepared scientific presentations and manuscripts, managed databases, and assisted in grant preparation

2012 – 2013 Consultant, Pan American Health Organization, Washington, D.C.
Responsibilities: Translated ProPAN 2.0 software technical specifications into Epi Info code, updated the software users' guide, provided training and technical support, developed dissemination materials, and assisted in the operationalization of WHO infant and young child feeding (IYCF) indicators

2011 – 2012 Research Assistant, Emory University Rollins School of Public Health, Atlanta, GA
Responsibilities: Contributed to data analysis for multiple child nutrition projects in Brazil and Bolivia, and assisted in the design of a maternal and child health, feeding, anthropometry, and housing survey

2012 Community Needs Assessor, Urban Health Initiative, Atlanta, GA
Responsibilities: Partnered with a low income community in Northwest Atlanta to address the issues of food security and health, and presented findings to community and stakeholders at a local food symposium at Emory University

2011 Intern, CARE India, Patna, India
Responsibilities: Collaborated with CARE staff to investigate infant and young child feeding practices in rural Bihar and conducted participatory research as well as content and skills trainings with front line health workers

2006 – 2010 Research Assistant and Lab Manager, Northwestern University, Evanston, IL
Responsibilities: Designed and conducted behavioral learning experiments in animal models to study memory, and analyzed data for reports; hired and trained students, assigned roles and duties and coordinated weekly lab meetings

2008 Collaborator, ShinAfrika Foundation, Kampala, Uganda
Responsibilities: Promoted a women's vocational school by putting on a drama for the community with the staff

TEACHING EXPERIENCE

2017, 2018 Teaching Assistant, Course: Food Technology and Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Responsibilities: Mentored masters level students, maintained course logistics, including scheduling and coordinating several offsite tours, and evaluated assignments and exams

2017 Teaching Assistant, Courses: Cellular Biochemistry of Nutrients and Nutrients in Biological Systems, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Responsibilities: Mentored masters level students, maintained course logistics, and evaluated exams

2016, 2017 Guest Lecturer and Teaching Assistant, Course: Assessment of Nutritional Status, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Responsibilities: Mentored masters level students, maintained course logistics, evaluated assignments and lectured on lessons learned in the field

2014 Guest Lecturer and Teaching Assistant, Course: Introduction to Epidemiology, Northwestern University Feinberg School of Medicine, Chicago, IL
Responsibilities: Mentored medical students and evaluated assignments and exams

2013 Teaching Assistant, Course: Introduction to Epidemiology, Northwestern University Program in Public Health, Chicago, IL
Responsibilities: Mentored Master of Science in Epidemiology and Biostatistics students, held lab sessions and evaluated assignments

PROFESSIONAL ACTIVITIES

Dietary Supplement Research Practicum Participant, National Institutes of Health - Office of Dietary Supplements, 2018

WISE (Women in Science and Engineering) Mentor, Garrison Ford High School/JHU, 2017

Invited Speaker, Wendy Klag Center for Autism & Developmental Disabilities Journal Club, Johns Hopkins Bloomberg School of Public Health, 2016

Member, American Society for Nutrition, 2016-

Invited Facilitator, Optifood Workshop WHO, Geneva, 2014

Invited Speaker, Northwestern Memorial Hospital Medicine Discovery Program, 2014

Invited Speaker, Sacred Heart School Interfaith Dialogue, 2009

Philanthropy Chair, Alpha Lambda Delta Honors Society, Northwestern University, 2006

LANGUAGES

English – native
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STATISTICAL PROGRAMS

Stata – proficient
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HONORS AND AWARDS

2018	Harry J. Prebluda Fellowship in Nutritional Biochemistry, Department of International Health, The Johns Hopkins Bloomberg School of Public Health
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2015	Feinberg Program in Public Health Teaching Assistant Award, Department of Preventive Medicine, Northwestern University
2011	Global Field Experience Award, Rollins School of Public Health, Emory University
2009	Darwin Scholarship, Northwestern University
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PUBLICATIONS

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VOLUNTEER WORK

2017 – 2018 Baltimore Free Farm, Baltimore, MD
Responsibilities: Sorting food for donations, preparing meals for the underserved, and maintaining the greenhouse

2015 – 2018 SOURCE, Baltimore, MD
Responsibilities: Volunteering with local community organizations, including The Family Tree to prevent child abuse and neglect and Project PLASE to support individuals experiencing homelessness

2013 – 2015 Curriculum Writer/ Project Manager, ECD (Early Childhood Development), Glenview, IL
Responsibilities: Oversaw the pooling of resources and assisted in the restructuring of the birth-three curriculum

2013 – 2014 Honorary Secretary, Aga Khan Education Board, Midwest USA
Responsibilities: Strategized and oversaw the execution of 20+ programs on early childhood, college planning, and continuing education for over 3,500 community members

2012 – 2013 Classroom Facilitator, ECD (Early Childhood Development), Glenview, IL
Responsibilities: Held weekly sessions with parents and children birth-six years of age implementing Montessori curriculum

2006 – 2013 Teacher, Religious Education Center, Glenview, IL
Responsibilities: Strategized and implemented three-hour weekly sessions for middle and high school students with a co-teacher